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BRAIN METABOLISM IN VIVO

1. The Distribution of Lesions Caused by Cyanide Poisoning, Insulin Hypoglycemia, Asphyxia in Nitrogen and Fluoroacetate Poisoning in Rats

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THE MECHANISMS by which selective lesions are produced in one tissue and not in another have often been subjects of investigation. Special interest has been directed toward the degree and the extent to which different parts of the nervous system are vulnerable to various forms of injury. Selective patterns of injury are frequently observed in this system both clinically and under experimental conditions. Asphyxia tends to damage the cerebral cortex, as does hypoglycemia, while carbon monoxide poisoning may damage the cerebral cortex and the corpus striatum in man and the hippocampus in some animals. A variety of toxic substances produce lesions in the white matter, the basal ganglions and peripheral nerves, and viruses show predilections for parts of the neuraxis. Certain dietary deficiencies cause damage in peripheral nerves and in the gray matter of the brain stem. Factors which might explain these patterns of injury, such as the relative richness of the regional blood supply, the selective permeability of the blood-brain barrier and the metabolic rate of certain regions and cells, have received attention.

Most efforts have been directed toward relating such regional susceptibility to a relatively poor blood supply.[1] White matter, for example, has about one-fifth the capillary length per unit volume of tissue that cerebral cortical gray matter has. The hippocampus and the substantia nigra are similarly poorly supplied. While these less well vascularized regions are often damaged by anoxia, they are probably not more generally vulnerable than the cerebral cortex, which is easily damaged,

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1. Alexander, L., and Putnam, T. J.: A. Research Nerv. & Ment. Dis., Proc. **18**:471, 1938. Finley, K.: Arch. Neurol. & Psychiat. **36**:118, 1936. Grenell, R. G.: J. Neuropath. & Exper. Neurol. **5**:131, 1946. Putnam, T. J.: J. Neurol. & Psychopath. **17**:193, 1937. Spielmeyer, W.: Medicine **10**:243, 1931 Uchimara, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **112**:1, 1928. Wolff, H. G.: Physiol. Rev. **16**:545, 1936; A Research Nerv. & Ment. Dis., Proc. **18**:29, 1938.

yet has a rich blood supply (fig. 1). It is evident that the capillary blood supply alone cannot be the factor responsible for vulnerability and that the metabolic demands of the tissue must be balanced against it.

This balance between metabolic demand and blood supply has been studied by Sharrer,[2] who noted that regions rich in synapses (neuropil), such as lamina I of the cortex, possess high metabolic rates and that the blood supply is usually correspondingly rich (fig. 1). He also

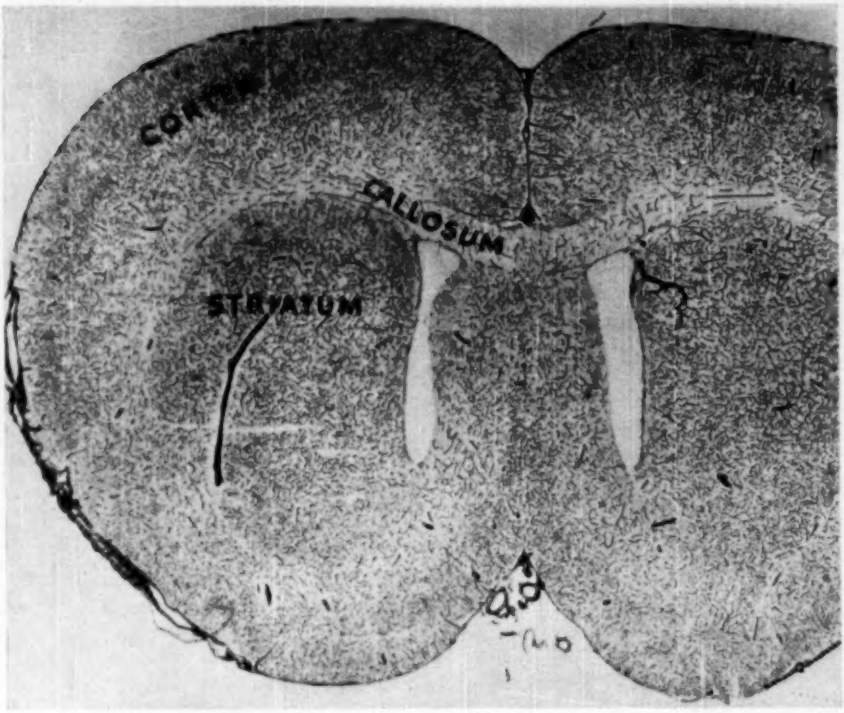

Fig. 1.—The brain of a white rat whose carotid arteries had received an injection of india ink. This frontal section of the anterior part of the cerebrum shows the relative vascularity of the cortex, the corpus striatum and the corpus callosum. (Low power magnification of a paraffin section 30 microns in thickness.)

postulated that the type of tributary vessels to an area and the relation of the capillaries to the cells may be modifying factors.

These and other observations concerning the relation of blood supply and selective injury have not offered a final solution of the problem, so

2. Sharrer, E.: Quart. Rev. Biol. **19**:308, 1944.

that some other approach is necessary. Vulnerability may depend to a large extent on the individual enzyme systems and other biochemical peculiarities of cells in different regions. Observations of the distribution of pathologic changes produced in the nervous system and other organs by substances which interfere with metabolism might therefore reveal useful information.

Pertinent to the problem of investigating enzyme systems and metabolic pathways in the living organism are certain experimental data concerning brain and nerve metabolism already gathered from both in vitro and in vivo sources. The adult intact brain appears to depend on glucose as a substrate [3] for its energy metabolism, and it consumes about one fifth of the body's oxygen supply.[4] It is therefore particularly susceptible to injury resulting from disturbances of carbohydrate metabolism and anoxia. It may not go into oxygen debt as skeletal muscle does, because it stores no appreciable glycogen. There is no evidence that it can utilize blood pyruvate and fats as seems to be the case in heart muscle,[5] possibly partly because glucose is the only energy substrate which is known to pass rapidly across the blood-brain barrier.[6] The protein and lipid turnover of the adult brain is slow compared with that in some other tissues.[7] Viewed as a homogeneous organ the brain is remarkably dependent on oxygen and glucose and therefore on an intact circulatory system. It is for that reason that studies of blood supply have held such importance.

These data apply only when it is assumed that the brain is a uniform tissue and when the organ is viewed as a whole, but there is evidence that different parts of the nervous system possess different forms of metabolism, both quantitatively and qualitatively. Quantitatively, the consumption of oxygen and glucose is greatest in the forebrain and least in the cord, and there is a corresponding descending gradient of susceptibility to injury in the neuraxis from before backward when these susbtances are lacking.[8] Qualitatively the metabolism of the brain stem and peripheral nerves seems to differ from that of some other parts of the brain, such as the cerebral cortex, as suggested by thiamine and

3. Quastel, J. H.: Physiol. Rev. **19**:135, 1939.

4. Kety, S. S., and C. F. Schmidt: Am. J. Physiol. **143**:53, 1945.

5. (*a*) Mulder, A. G., and Crandall, L. A.: Am. J. Physiol. **137**:436, 1942. (*b*) Olson, R. E., and Kaplan, N. O.: J. Biol. Chem. **175**:515, 1948. (*c*) Olson, R. E.; Miller, O. N.; Topper, Y. J., and Stare, F. J.: ibid. **175**:503, 1948. (*d*) Olson, R. E.; Pearson, O. H.; Miller, O. N., and Stare, F. J.: ibid. **175**:489, 1948.

6. Klein, R., and Olsen, N. S.: J. Biol. Chem. **167**:1, 1947. Mayer-Gross, W., and Walker, J. W.: Biochem, J. **44**:92, 1949.

7. Fries, B. A., and Chaikoff, I. L.: J. Biol. Chem. **141**:479, 1941. Hyden, H.: Acta physiol. Scandinav. (supp. 17) **6**:1, 1943. Sperry, W. M.; Waelsch, H., and Stoyanoff, V. A.: J. Biol. Chem. **135**:281, 1940.

8. Ferris, S., and Himwich, H. E.: Am. J. Physiol. **146**:389, 1946.

possibly other B complex deficiences. Likewise, selective lesions developing in the brain stems of monkeys after certain quinoline compounds [9] were administered suggest that qualitative biochemical differences exist within different regions of the brain.[10]

Still other data point to qualitative metabolic differences. The brains of some newborn animals possess metabolic pathways which utilize carbohydrate both aerobically and anaerobically.[11] By virtue of the anaerobic process they are able to withstand considerable periods of anoxia. This capacity to utilize carbohydrate anaerobically disappears soon after birth, and with this qualitative change a quantitative shift occurs. Whereas at birth the highest rate of glycolysis occurred in the lower brain stem, in adult life the maximal rate of metabolism shifts to the forebrain. The nature of this change of brain metabolism is unknown but may bear some relationship to the postnatal increase of cytochrome oxidase.[12]

These observations pose a number of questions which it is the purpose of these and subsequent experiments to answer. Is glucose virtually the sole energy substrate for the adult nervous system, or do peripheral nerves and certain brain stem nuclei utilize other substances as well? What are the relative importances of blood supply, blood-brain barrier and type of tissue metabolism in determining selective vulnerability of parts of the nervous system? What differences of metabolism are there among neurons, their myelinated fibers, the glia and the endothelium of blood vessels which might govern the distribution of lesions? How does the brain compare with other tissues in regard to susceptibility to specific metabolic injuries?

A program of systematic studies has therefore been undertaken to determine the distribution of, and the underlying mechanisms concerned with, pathologic changes produced by enzyme inhibitors and other agents which interrupt metabolism in the nervous system and other organs. Although many variables and secondary effects may be involved in dealing with a total living organism rather than with an isolated tissue in the chemical laboratory, it is certain that much may be learned about the metabolic pathways actually utilized by various tissues and parts of the nervous system in the normal living organisms and the processes involved in the development of selective lesions. There is ample justifi-

9. Richter, R.: J. Neuropath. & Exper. Neurol. **8**:155, 1949.

10. Preliminary studies in this laboratory with 8-(-3-diethylaminopropylamino)-6-methoxyquinoline (plasmocid) show that in the rat the gasserian ganglion cells, the heart and especially the skeletal muscle rather than the brain stem are selectively damaged.

11. Himwich, H. E., and others: Am. J. Physiol. **135**:387, 1942; Proc. Soc. Exper. Biol. & Med. **46**:553, 1941.

12. Page, I. H.: Chemistry of the Brain, Springfield, Ill., Charles C Thomas, Publisher, 1937, p. 303.

cation for testing in vivo the results of metabolic studies conducted in vitro. Meyerhof [13] has recently shown that several phases of brain metabolism in vitro can be altered very much at will by the biochemist, depending on the form of the tissue used—homogenates, slices, etc.—and the substrates provided. Further, there are several practical observations which show that the intact nervous system behaves metabolically quite differently from the tissue isolated in vitro. A number of experiments, especially the more recent ones of Kety and Schmidt,[4] have shown that the living, intact brain may utilize several times as much oxygen as does brain in vitro. Himwich and Saunders [14] have shown that in vitro brain will rapidly detoxify approximately its own weight in sodium cyanide, whereas in vivo a few milligrams will so interrupt its integrated function that death of the whole organism ensues rapidly. In vitro brain metabolizes fructose, succinate, lactate and pyruvate, but for practical purposes only glucose will pass rapidly across the blood-brain barrier to relieve hypoglycemic shock.[6] These findings make it not only justifiable but necessary to test many of the in vitro results in the intact animal.

In order to carry out these studies a number of substances have been used or are in process of investigation. Results have already been obtained by the use of insulin, which deprives the brain of glucose; cyanide, which in small quantities stops intracellular oxygenation through the cytochrome complex by inhibiting cytochrome oxidase; simple asphyxia in nitrogen, and poisoning with fluoracetate, which is believed by some [15] to interfere with certain phases of pyruvate metabolism through the interruption of acetate metabolism. The patterns of lesions produced in the brain and other organs of animals by these four agents constitute the material to be reported in this paper.

METHODS

General Outline of Experiments.—The experiments, which will be given in further detail under their respective headings, consisted in administering to white rats sublethal doses of sodium cyanide, insulin or sodium fluoroacetate in single or repeated doses, or in partially asphyxiating them repeatedly in nitrogen. The fundamental aim was to produce the maximal damage possible just short of killing the animal immediately and thus to produce destructive lesions in those tissues which were most vulnerable to the agent. As there were variations in the physiologic reactions and the behavior responses of the animals, the dosages were varied

13. Myerhof, O., and Geliazkowa, N.: Arch. Biochem. **12**:405, 1947. Myerhof, O., and Wilson, J. R.: ibid. **14**:71, 1947.

14. Himwich, W. A., and Saunders, J. P.: Am. J. Physiol. **153**:348, 1948.

15. (*a*) Bartlett, G. R., and Barron, E. S. G.: J. Biol. Chem. **170**:67, 1947. (*b*) Chenoweth, M. B., and Gilman, A.: J. Pharmacol. & Exper. Therap. **87**:90, 1946; (*c*) Bull. U. S. Army M. Dept. **7**:687, 1947. (*d*) Ward, A. W.: J. Neurophysiol. **10**:105, 1947.

to attain this maximal sublethal effect in each case. The type of injury induced may be classed as acute, for each episode was of short duration and was not repeated over a long period. Several dozen rats were used in each group of experiments in order to establish a general pattern of reaction and to minimize the results of individual variations. They were males and females weighing 220 to 300 Gm. usually, kept at room temperature in wire cages and fed purina® dog chow and water ad libitum except as noted in the insulin animals. The strain used was an inbred one that was developed at the Harvard University Biological Laboratories during the past twenty years and originally derived from Wistar stock.

When a suitable interval had elapsed after the metabolic injury—usually two or more days in order to allow morphologic evidence of destructive lesions to develop—the animals were killed and examined pathologically. The brains were studied microscopically by interrupted serial sections, and necrotic lesions, when they occurred, were mapped according to Krieg's [16] topographic charts of the rat's brain. A record of the exact areas affected was kept, but available space does not permit their being recorded here in detail. Although emphasis was laid on the nervous system, the other organs were studied microscopically whether lesions of the brain were produced or not. Heart, liver, kidney, spinal cord and sciatic nerve were examined from virtually all animals. From a majority of animals the following organs were studied: eye, pituitary gland, organs of the neck, trigeminal nerve and ganglion, vertebra with marrow, skeletal muscle, thymus, lung, adrenal gland, pancreas, small gut, spleen and testes or ovaries. The sections of the spine frequently afforded a view of the nerve roots and the spinal and sympathetic ganglions, as well as of the aorta. The sections of the organs of the neck showed the thyroid gland, the esophagus, the trachea and associated structures, and in about half of them a parathyroid gland appeared. Sections were only occasionally made of seminal vesicles, stomach, skin, salivary glands or specific lymph nodes, but lymphatic tissue was well represented in the gut, the thymus and the sections of the neck organs, as well as in the spleen.

Usually the animals were killed by severing the thoracic part of the spine, and the carotid arteries were immediately perfused with fixative, most often Bouin's fluid. The tissues were embedded in paraffin, and sections were stained routinely with hematoxylin and eosin. Although this stain was the most useful, Bodian's strong protein silver, phosphotungstic acid-hematoxylin, reticulum and iron-hematoxylin myelin stains were also used on the sections of nervous tissue. A combination of thionine and a light hematoxylin stain was also useful. Examination of organs and tissues other than the nervous system was carried out usually by fixing in Bouin's fluid, embedding in paraffin and staining sections with hematoxylin and eosin. Fat and glycogen stains were employed in some instances in the study of the fluoracetate animals.

Cyanide Experiments.—The lethal dosage of sodium cyanide for 50 per cent ($L.D._{50}$) of young adult white rats of the strain used was found to be close to 5 mg. per kilogram in a dilution of 1:1,000 by the intraperitoneal route. There were small variations depending on the dilutions used. With a 1:1,000 dilution survival after 6 mg. per kilogram occurred rarely, and with a 1:500 dilution 5 mg. per kilogram often produced death. When it was found that usually doses had to be given rapidly over a short period (hours) to produce lesions of the brain, a nonlethal but severely convulsive dose of 3 mg. per kilogram was usually

16. Krieg, W. J. S.: Quart. Bull., Northwestern Univ. M. School **20**:199, 1946; J. Comp. Neurol. **84**:221 and 277, 1946.

employed, in a dilution of 1:1000. There is such a small margin between a dose which will kill a rat in less than two minutes and one which will just fail to kill it and yet produce lesions in the brain that it required the sacrifice of a number of animals to determine the optimal effective dosage. Only rarely did a single dose cause destructive lesions of the brain.

The method of administering the cyanide and the immediate effects obtained may be briefly illustrated here in composite terms of typical experiments. The rat was given the calculated amount of cyanide intraperitoneally by means of a syringe and hypodermic needle. In about thirty seconds it became uneasy, then exhibited incoordinated movements and usually within a minute became convulsive or comatose. The convulsions were characterized by bursts of rapid jerking extensions and flexions of the hindlimbs lasting for a few seconds and occurring intermittently for a minute or two. Between these outbursts the animal was relaxed, or sometimes the whole spine and the limbs were extended. For a period of ten to thirty minutes after the injection of the cyanide the animal was virtually comatose, occasionally showing small bursts of convulsive movements, over-all extensor spasms or coarse shivering. A corneal reflex was usually absent during this stage. The heart rate was rapid and regular during the phase of acute poisoning, and shock or circulatory failure did not develop. Sometimes a brief period of apnea lasting fifteen to thirty seconds followed the first convulsive seizure, but cyanosis did not occur in animals which survived; otherwise respirations were uniformly rapid. As soon as the animal showed signs of recovery, another dose of cyanide was given, and the convulsive state was repeated. There was a general uniformity of response on the part of animals, but, within a range, individual variations were frequent. Rarely the animal did not respond typically to one of the doses in a repeated series, becoming only partially comatose. As the experiments progressed it became evident that lesions could be produced with a fair degree of certainty if over a period of hours the rat was given a dose every time it showed signs of recovery. A group of 4 or 5 animals were usually treated simultaneously and the doses adjusted in frequency to the requirements of the individual rat. After the period of poisoning they appeared to recover rapidly and completely within a period of one to six hours. Although they were closely observed and a rough neurologic examination was made, they showed no distinctive patterns of behavior or neurologic disturbances which could be regularly correlated with the lesions. This was probably because most of the lesions were not extensive enough and because detailed studies of neurologic function and behavior were not carried out. One animal in which the anterior corpora striata and corpus callosum were severely damaged showed moderate incoordination in walking, could not walk along a narrow ledge without slipping off and appeared to walk with the limbs in a more extended posture than normal.

Insulin Experiments.—The conduction of these experiments was generally analogous to that of the cyanide experiments. The rats were given crystalline insulin intraperitoneally in single or repeated doses ranging from 2 to 20 U. S. P. units, the sole aim being to produce sublethal hypoglycemia. In most instances they had fasted for a day or more, for it is usually difficult to induce insulin hypoglycemia in rats that have access to food. Although the animals did not always respond to the insulin, in most instances the result was typical. About three to four hours after the insulin was given, the rat became sluggish, then stuporous and finally comatose or convulsive. During this phase the heart beat was rapid and regular. Circulatory shock or failure did not develop in those animals that survived, but in some animals that did not survive terminal pulmonary edema occurred. Respirations were generally rapid in the preconvulsive stage, then slower as the comatose condition was reached. This sequence usually developed in fifteen to thirty

minutes, convulsions or coma and complete flaccidity marking the prelethal stage. At this point 5 per cent dextrose was injected intraperitoneally in the amount of 2 to 5 cc., with recovery usually in a few minutes. Occasionally the administration of dextrose had to be repeated when an animal lapsed into the hypoglycemic state. It is established that in rats the convulsive state corresponds to zero blood glucose,[17] but what the duration of this absolute state was in each of these experiments is not known, although it probably was a matter of minutes. However, except for a few atypical reactions, it is estimated that the total duration of the severe phases of depressed blood sugar lasted about fifteen to thirty minutes in each experiment.

Asphyxia Experiments.—In these experiments rats were immersed in a closed 3 liter jar containing nitrogen until convulsions and coma ensued. As in the previous experiments, the aim was to produce the maximal injury short of killing the animal. The immersion was repeated at intervals ranging in number from two to sixty, usually from forty to sixty. The rats were dropped into the jar, through which nitrogen flowed at a rate of 8 to 15 liters per minute. In about forty seconds the animal commenced rapid running movements, which were followed by rapid, jerky, generalized convulsions. Within sixty to eighty seconds of asphyxiation, coma and apnea developed, and the animal was immediately removed from the jar. At this stage the heart beat became too rapid to count and sometimes for a few seconds thereafter could scarcely be palpated. With a few inspirations of air, the heart beat returned rapid and strong. Pulmonary edema rarely occurred. Occasionally artificial respiration was instituted briefly to restore breathing. Intervals of about forty seconds to three minutes were allowed to lapse between bouts of asphyxia in different experiments. Thirty animals were used in this series. Although general weakness, poor equilibrium and spasticity of the hindlegs frequently developed after the course of asphyxia, these symptoms lasted only an hour or two and within a few hours complete recovery had seemingly occurred; beyond these transient symptoms none of the animals showed measurable residual disease.

Fluoracetate Experiments.—These were conducted in a manner generally similar to that used in the foregoing experiments. They consisted of administering sodium fluoroacetate to male and female white rats in single or repeated doses, then killing them from two days to three weeks later and studying the nervous system and other tissues histologically. The drug was given intraperitoneally in doses of 1 to 3 mg. per kilogram in a 1:1,000 dilution. Three milligrams was usually fatal while 2 mg. often failed to kill.

The reactions of the animals to the fluoracetate as given by this method warrant a description in view of the variable species response to this compound (Chenoweth and Gilman [18b]). About three hours after the compound was administered, the rat became restless, and examination revealed a rapid heart rate of about 150 or more beats per minute with occasional dropped beats, frequently varying to a slower or a more rapid rate, too fast to count. During this phase the convulsive state developed without warning symptoms, the whole episode lasting about a minute. The animal suddenly commenced rapid running movements, which lasted a few seconds and ended in an explosive extension of all four limbs. This extension was of sufficient force in some instances to propel the animal 8 or 10 feet (about 2.5 to 3 meters) through the air from a bench, or, when the animal was in a cage, to knock aside the wire top of the cage, which weighed several ounces. With the onset of the extensor spasm of the limbs, the head became rigidly flexed on the

17. Dotti, L. B., and Hrubetz, M. C.: J. Biol. Chem. **113**:141, 1936.

chest, and all the skeletal muscles became rigid, in a degree somewhat reminiscent of rigor mortis. Respiratory movements, of course, ceased. After about thirty or forty seconds of rigidity, the animal relaxed and temporarily resumed fairly normal activity. Despite the suddenness of the onset of convulsions, several observations were made on the action of the heart at this stage. In 3 or 4 instances the animals were being handled when the convulsions began; the cardiac rate was rapid, the beat strong and the rhythm somewhat irregular. In a total of about 8 animals the heart was palpated during the phase of muscular rigidity. In each instance the heart beat could not be felt during this phase, but just as the animal began to relax and resume activity, the heart beat came back rapidly and continued as before the convulsions.[18]

Residual neurologic disease of an irreversible nature was as difficult to evaluate in these living animals as it was in the cyanide and insulin experiments. As a rule, the animals were killed before a sufficient period had elapsed for reversible effects to subside. The convulsive episode usually occurred only once, occasionally twice or three times, whether the fluoroacetate was given in single or multiple doses. In animals that received small daily injections for several days, the convulsions developed as a rule only once. When the dose was 2 mg. or more, the animal receiving it usually became moderately weak and irritable within twenty-four hours, but cyanosis and dyspnea were not seen. Those receiving 1 mg. per kilogram daily were seemingly little affected.

RESULTS

Pathologic Changes.—Few tissues are so easily altered by agonal changes in the intracellular and extracellular fluids, by postmortem autolysis and by fixation as is the brain. Therefore, in these experiments necrosis of nerve cell bodies, myelinated fibers or the parenchymal ground substance was the only lesion considered significant in estimating the destructive effect of the various agents in the nervous system. These are illustrated in the photomicrographs. Proliferation of vascular endothelium and brain phagocytes was also seen, but this was not per se a destructive lesion. Hyperchromatic and pale-staining neurons—in regard both to cytoplasm and nuclei—were often found in experimental animals at varying intervals after injury, but they were seen with equal frequency in abundant control material. This irregularity of staining sometimes appeared asymmetrically in paired nuclei of the brain stem, or it occurred symmetrically in corresponding cortical regions. The Purkinje cells nearly always showed irregular staining characteristics in control and experimental animals. Postmortem autolysis, especially in animals fatally injured by one of the agents, also altered the staining of nervous tissue markedly, even within an hour, so that the findings in animals discovered dead were discounted.

In these experiments the destructive lesions of the brains ranged from necrosis of scattered neurons to complete dissolution of a region, not unlike that seen in infarction. The lesions were nearly always symmetrically bilateral. The cyanide produced damage in the cerebral cortex, the corpus striatum (especially the white matter), the corpus callosum, the substantia nigra and the hippocampus, for the most part, while insulin most often damaged the cortex, the corpus striatum and the hippocampus. Asphyxia caused necrosis of the cerebral cortex, the corpus striatum and the substantia nigra only. Fluoroacetate produced minimal cortical

18. Data on possible variations of the heart rate over a period of days were not gathered.

brain damage. The peripheral nerves, the trigeminal, spinal and sympathetic ganglions and the spinal cord were not injured. The mildest form of brain damage was evidenced by death of nerve cell bodies. The cytoplasm became eosinophilic, greatly reduced in volume and poorly outlined. The nucleus was similarly reduced in size, and it became homogeneously basophilic and poorly outlined (figs. 5, 6 and 8). Such morphologic evidence of necrosis became fully developed in less than twenty-four hours. Necrosis of white matter was evidenced early by swelling, disintegration and loss of staining properties and was found in association with greater or lesser damage to some part of the gray matter. The fatty myelin part of the fibers broke down rapidly within twenty-four to forty-eight hours and the axis-cylinders, as shown by Bodian stains, disintegrated somewhat less rapidly, so that "demyelinization" in these experiments was largely a matter of death of whole myelinated nerve fibers (figs. 2 and 3).

In the mildest lesions—death of scattered neurons—there was little vascular response, and phagocytosis or edema of neurons did not occur. However, in virtually all animals that were exposed to the injurious agents and that survived twenty-four hours or longer, whether parenchymal necrosis occurred or not, there was some increase in number of microglia throughout the brain, especially in the cortex, the corpus striatum, the thalamus and the midbrain. Also, in the middle cortical layers and the corpus striatum, endothelial cells became prominent by virtue of being swollen or sometimes actually increased in numbers. In more severe damage, with necrosis of nerve cell bodies, fibers and ground substance, the vascular and phagocytic response was more prominent, and swelling of cellular elements and interstitial edema occurred in the necrotic regions. Mitotic figures were frequent in the microglia, after a day or two, in the more severe degrees of injury. In the few animals allowed to survive for three weeks with severe lesions, gliosis developed around areas of necrosis. In a few instances eosinophilic dead neurons continued to be visible for as long as three weeks in the cerebral cortex where injury had been very slight, but in the corpus striatum under similar circumstances necrotic neurons usually disappeared or became very faint within a few days without exciting phagocytosis. Thus it was found advisable usually to examine the animals within a few days after injury in order readily to locate mild damage.

The severity of parenchymal damage of the brain was approximately estimated in all animals in terms of 1, 2 and 3 plus. If a few scattered nerve cell bodies had died in a given region, the damage was recorded as 1 plus, for this seemed to represent the least degree of destruction, though in cyanide animals the white matter of the corpus striatum appeared to be damaged as readily. More extensive destruction of neurons was graded 2 plus. In the corpus striatum this degree of damage was often accompanied by necrosis of the bundles of myelinated nerve fibers in cyanide animals, this being less conspicuous in other experiments. In some of the cyanide animals the myelinated fibers of the corpus callosum were destroyed to varying degrees, and this destruction was estimated by a similar manner of grading. Three plus injury was estimated when the lesion was completely destructive, and in general the histologic sequence of events which developed was similar to that seen in infarction. Thus this approximation or grading of lesions was based on severity of injury, not on the extent of the lesion. For example, one animal might have 3 plus necrosis of the substantia nigra only, while another might sustain 1 plus cortical damage extending over a large surface but involving only scattered neurons.

Severity of lesions in other organs was also estimated on a rough scale of 1, 2 and 3 plus.

One possible complicating factor which arose in the histologic evaluation of lesions of the brain in these experiments, and may again in others to follow, requires mention. In a few animals treated with a cyanide, insulin and other drugs,[19] disseminated acute inflammatory lesions occurred in several organs, including the nervous system. In these experiments they occurred in 1 animal of the cyanide and 2 of the insulin series but never in the asphyxia or fluoroacetate animals or controls. The encephalitic lesions consisted of focal nodules of elongated microglial cells, monocytes, lymphocytes and rarely polymorphonuclear leukocytes, and they occurred in sparse numbers anywhere in the central nervous system, including the cord. In other organs the corresponding lesions were a few small foci of necrosis in the spleen, the lung and the kidney. The etiologic agent was not determined. The inflammatory cells in these lesions exceeded greatly the response seen in the metabolic necrotic lesions, and no difficulty was experienced in dis-

TABLE 1.—*Summary of the Principal Lesions of the Nervous System Caused by Cyanide Poisoning in 22 Rats*

	Animals in Which Region Indicated Showed Lesions
Corpus striatum	19
Corpus callosum	5
Hippocampus (pyramidal cell layer)	8
Cerebral cortex	10
Substantia nigra	7

TABLE 2.—*Protocols of 4 Representative Rats Receiving Sodium Cyanide*

Rat	Dose and Duration of Treatment	Corpus Striatum	Hippocampus	Cortex (by Area)*	Substantia Nigra	Corpus Callosum
48R40	5 mg. per kilogram 6 times in 7 days	1+	0	6, 9, 12, 14, 17, 18a, 23 and 28 (3+); 32, 35, 36, 41 and 51b (3+)	1+	2+
48R43	5 mg. per kilogram 7 times in 6 days	0	0	0	2+	0
48R12	6 mg. per kilogram once	1+	0	0	0	0
48R89	3 mg. per kilogram 14 times in 2 days	2+	Pyramidal cell layer 1+	51b (2+)	0	0

* The numbers indicate cortical areas according to Krieg.[18]

tinguishing between the two in the early acute phases. They are mentioned here for two reasons: 1. Apparently the metabolic damage sustained by these experimental animals renders them occasionally susceptible to secondary infection, as shown by the recent nature of the lesions in animals killed soon after experiments were performed. 2. In evaluating older lesions, as when animals are allowed to survive for three weeks or longer, the histologic appearance of a completely necrotic region caused by encephalitis might be hard to distinguish from a similar one caused by metabolic injury. Although this offers little difficulty in the present acute experiments, it must be carefully considered in long-standing chronic experiments in which inhibitors of enzymes are employed.

Cyanide Experiments (tables 1 and 2).—In addition to preliminary experiments, 53 animals were repeatedly poisoned with sodium cyanide to the point of convulsions and coma over a period of hours or in some instances days. Seventeen died in the acute stage of poisoning; 14 survived without lesions developing; in the remaining 22 destructive lesions of the brain of varying severity occurred.

19. Fluoride and iodoacetate were used.

The lesions of the brain are summarized in table 1, and sample protocols are given in table 2. The injury occurred most often in the corpus striatum in its anterior regions. Damage seldom extended back into the putamen and the globus pallidus. As already noted, both gray and white matter were affected, nerve cell bodies being generally the most vulnerable. The cortex of the lateral surfaces of the cerebral hemispheres, usually the outer laminas of neurons, were affected only less often than the corpus striatum. Lesions of the cortex, though bilateral, were variable in degree and extent, sometimes being rather focal, other times being less sharply defined and involving the outer cell layers fairly extensively. Neurons

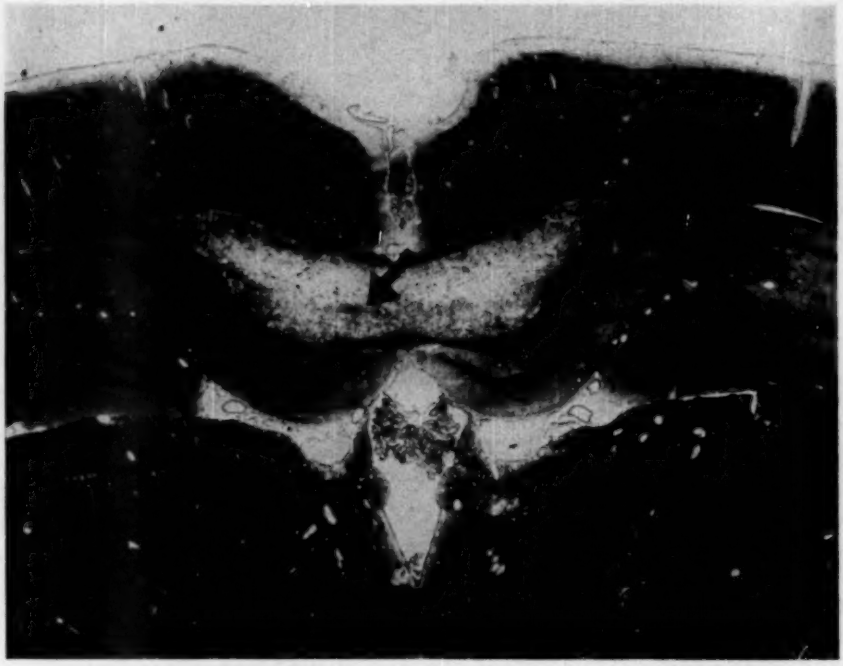

Fig. 2.—Cyanide poisoning. Severe necrosis of the posterior part of the corpus callosum appears as the light area indicated by the arrow. (Iron-hematoxylin stain for myelin; low power.)

in the fifth and sixth laminas were not as often involved. Less often damaged were the pyramidal layer of neurons in the hippocampus, the corpus callosum and the substantia nigra. Necrosis of the myelinated fibers of the corpus striatum was more conspicuous in these cyanide than in the insulin and asphyxia experiments. Only once was the optic nerve affected. In 3 animals with severe lesions of the brain, small areas of the folia of the lateral cerebellar hemispheres were damaged. This consisted of necrosis of parenchyma involving the molecular and granular zones, Purkinje cells and white matter.

Fig. 3.—Cyanide poisoning. This is an area of figure 2, to show the corpus callosum in detail. Mitotic figures still occur in phagocytes two weeks after the initial injury. (Phosphotungstic acid–hematoxylin; × 250).

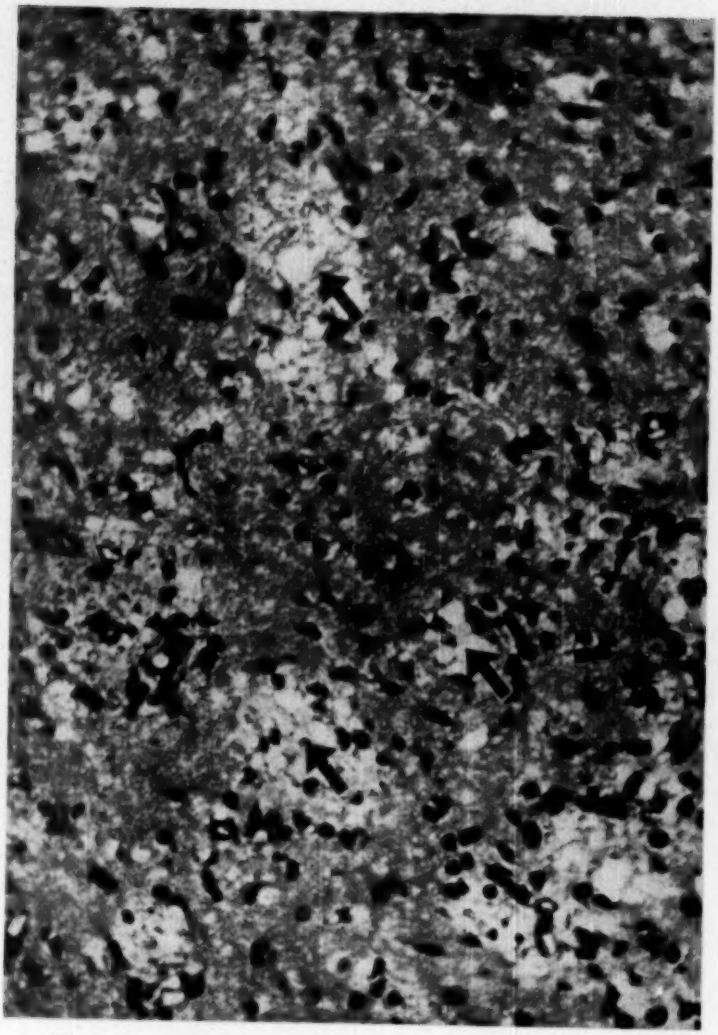

Fig. 4.—Cyanide poisoning. Acute necrosis of the corpus striatum, especially involving the bundles of myelinated fibers, in rat 48R89 (table 2). (Hematoxylin and eosin; × 250.)

In no instance was the brain stem (except the substantia nigra) or the thalamus damaged, these regions being strikingly spared even in those animals in which adjacent regions such as the corpus striatum and the substantia nigra sustained extensive 3 plus injury. In 2 animals, some cells in the nuclear layer of the retina were necrotic, but the rod cell nuclei were not affected.

All combinations of the lesions occurred. Considering all of the animals, one might assume that the corpus striatum and the cerebral cortex are the most vulnerable because they were most often damaged, but in 2 rats necrosis appeared in the substantia nigra only, and in another severe focal cortical damage but no other lesions developed. A close correlation of dosage and lesions is therefore not presently possible. This serves to point up the narrow margin between a sublethal dose which will produce no neurologic lesions and one that is lethal.[20]

TABLE 3.—*Summary of Principal Lesions of the Nervous System Caused by Insulin Hypoglycemia in 23 Rats*

	Animals in Which Region Indicated Showed Lesions
Corpus striatum	12
Cerebral cortex	20
Hippocampus (pyramidal cell layer and fascia dentata)	0

TABLE 4.—*Protocols of 3 Representative Rats Receiving Insulin*

Rat	Dose and Duration of Treatment	Corpus Striatum	Hippocampus	Cortex (by Area)*
48R41	20 units twice, 6 days apart, 48 hr. post absorption	1+	Pyramidal cell layer 3+	2, 13, 14 (2+)
49R16	4 units 24 hr. post absorption	2+	0	2, 13, 14, 51a, 51b (1+)
49R22	4, 10 and 20 units 2 days apart, 24 hr. post absorption	0	0	13, 14, 20, 51b (1+)

* The numbers indicate cortical areas according to Krieg.[16]

Examination of other organs showed no consistent pattern of pathologic changes and none that were destructive. In the thyroid gland the cells of the acini often became slightly taller than cuboidal, and the cytoplasm adjacent to the colloid was vacuolated. Destructive lesions did not occur in the heart, the liver, the kidneys, the testes or the ovaries.

Insulin Experiments (tables 3 and 4).—Forty-one rats were used, and in 23 of these necrotic lesions developed in the brain; 3 recovered completely so far as the nervous system was concerned, and 15 died during the hypoglycemic shock. The lesions of the brain showed similarities to, and differences from, those observed in the cyanide experiments. The cerebral cortex and the corpus striatum were damaged, sometimes severely, but the corpus callosum, the substantia nigra and the cerebellum were never involved. The white matter of the striatum was not conspicuously damaged as it was in some of the cyanide animals. The neurons of the fascia dentata, as well as the pyramidal layer of the hippocampus, were extensively necrotic in several animals, whereas in the cyanide animals destruction occurred exclusively in the region of the pyramidal cells. Also, the thalamus

20. Hurst, E. W.: Australian J. Exper. Biol. & M. Sc. **20**:297, 1942.

Fig. 5.—Insulin hypoglycemia. Acute necrosis of pyramidal cells of the hippocampus, evidenced by presence of small, dark nuclei and virtual absence of cytoplasm. At lower right some normal neurons remain. (Hematoxylin and eosin; × 250.)

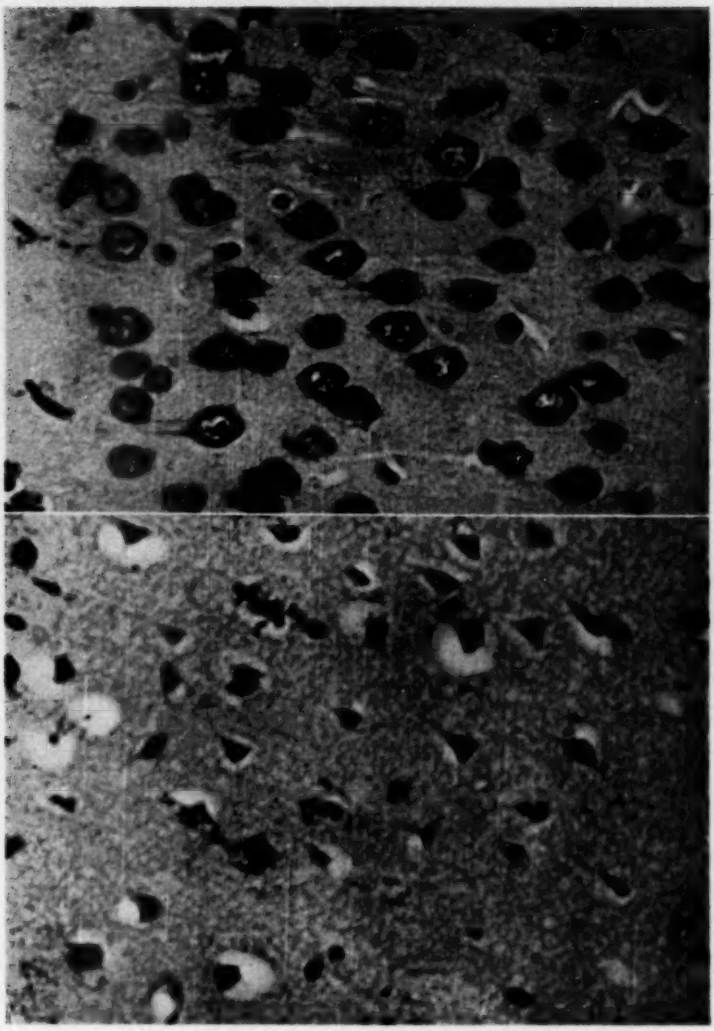

Fig. 6.—Insulin hypoglycemia. Necrotic neurons in outer cortical layers compared with undamaged cells in another region. (Thionine and hematoxylin; × 450.)

showed rare necrotic neurons in occasional instances, as did the septal nuclei adjacent to the corpus striatum. No strict correlation of dosage and brain damage could be established, but it is noteworthy that necrotic lesions, sometimes severe, followed single bouts of hypoglycemia.

Examination of other organs revealed some lesions. In a single instance necrosis of some neurons was noted in the nuclear layer of the retina. In about half the animals the heart was damaged, usually very slightly but in one instance rather

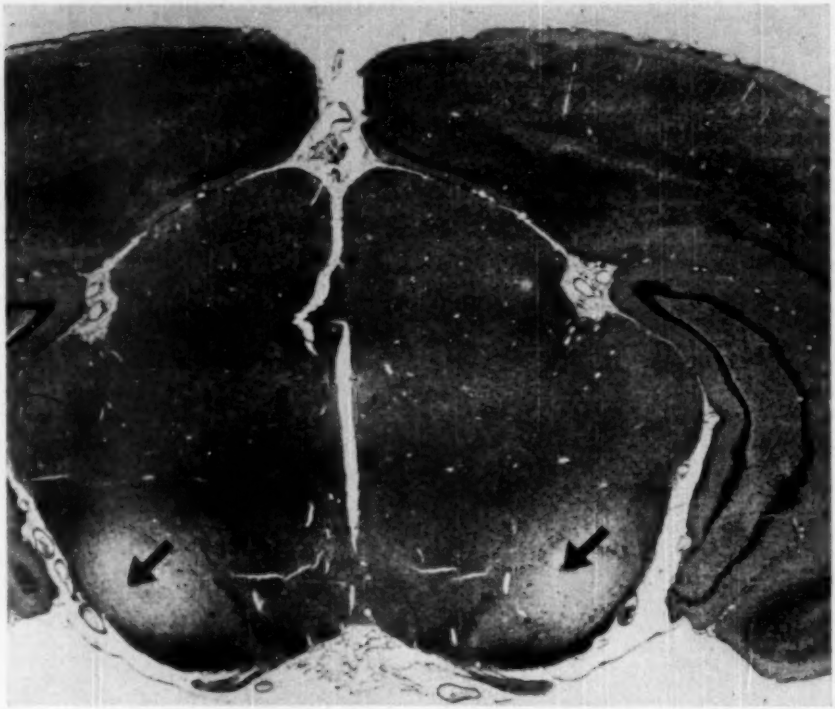

Fig. 7.—Asphyxia in nitrogen. Acute necrosis of the substantia nigra in rat 49R45 (table 6). The scattered necrotic cortical neurons in areas 17 and 18a are not visible at this magnification. (Hematoxylin and eosin; low power.)

severely. This mild damage also occurred in several rats with no lesions of the brain. The cardiac changes were similar in nature to those which were often severe in the fluoroacetate animals (see later section) and which were characterized by accumulations of mononuclear cells and necrosis of muscle fibers. For the most part, these lesions in the insulin animals were focal, infrequent and evident more by virtue of the accumulation of monocytes than by the muscle necrosis, which was usually minimal. The liver of 1 animal showed scattered small foci

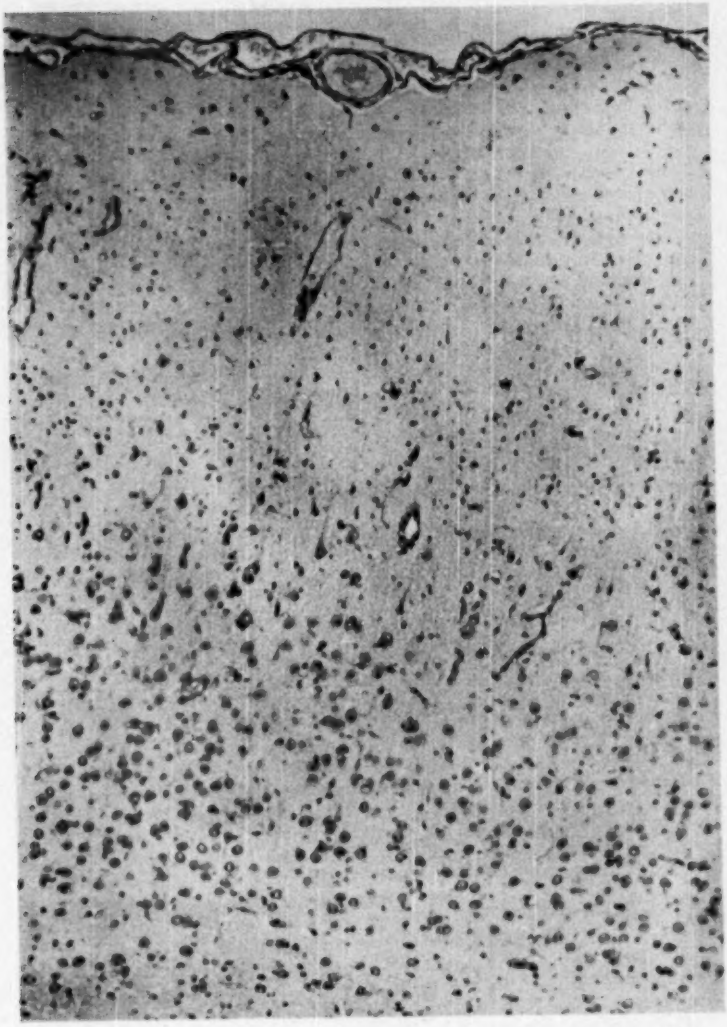

Fig. 8.—Asphyxia in nitrogen. The outer layers of neurons are necrotic; those in the deeper layers are spared. (Thionine and hematoxylin; × 150.)

of central necrosis, but this never occurred in the others. Frank necrosis was not present in the kidneys, but in a few instances vacuolation of proximal convoluted tubules and pyknosis of the nuclei of some cells of the loops of Henle were seen. Testes and ovaries were always spared.

Asphyxia Experiments (tables 5 and 6).—Thirty animals were asphyxiated, and in 16 the brain was damaged in consequence. Of the remaining ones, 3 died during the process of asphyxiation, and 11 recovered without lesions of the nervous system.

The cerebral cortex, the corpus striatum and the substantia nigra were the only regions involved; the hippocampus was spared, in contrast to the cyanide and insulin experiments, and selective damage of white matter was not present. In some animals the cortical necrosis was extremely severe and extensive. No destructive lesions were found in any other organs except the heart. With only one or two exceptions, the hearts of animals that survived showed mild lesions, whether

TABLE 5.—*Summary of Principal Lesions of the Nervous System Caused in 16 Rats by Asphyxia in Nitrogen*

	Animals in Which Region Indicated Showed Lesions
Corpus striatum	13
Cerebral cortex	14
Substantia nigra	6

TABLE 6.—*Protocols of 3 Representative Rats Asphyxiated in Nitrogen*

Rat	Duration of Asphyxia	Corpus Striatum	Cortex (by Area)*	Substantia Nigra
49R45	56 bouts in 5 hr.	1+	3, 4, 17, 18a, 51a (1+) 6, 35, 40, 41 (2+) 8, 10, 12, 14 (3+)	2+
49R61	60 bouts in 3½ hr.	0	0	1+
49R49	9 bouts in 40 min.	1+	2, 13, 14 (1+)	0

* The numbers indicate cortical areas according to Krieg.[14]

or not damage of the brain developed. These were generally similar to those seen in the insulin animals but were never as severe or extensive as those in some fluoracetate rats. Thus the heart was more consistently but never as seriously susceptible to anoxia as the brain. In renal tubule cells, changes like those in the insulin experiments developed occasionally, but necrosis was not seen.

As in the cyanide experiments, there was a narrow margin between a degree of asphyxia sufficient to kill the animal and one that would just fail to produce lesions of the brain; several animals recovered completely after being immersed in nitrogen forty to sixty times, while in a few instances relatively few bouts of asphyxia resulted in minimal to moderately severe lesions.

Fluoracetate Experiments (table 7).—Thirty-nine animals were used in the experiments. Of these, 33 survived a sufficiently long time (twenty-four hours or more) for lesions to become visible, while 6 died in the acute phase of poisoning. Three additional animals were prepared for glycogen and fat studies as noted later.

The most striking lesions occurred in the heart and the testicle, while the brain was not often involved and never seriously. The kidney and the liver were

damaged in some instances, but in many cases escaped destructive damage. The retina was damaged only once, in a manner similar to that of the cyanide and insulin animals. The vertebral marrow occasionally showed mild general hyperplasia. Hemosiderin occurred in sinusoidal lining cells in the liver and in increased amounts in the spleen in 3 animals, but data were not obtained on the peripheral blood in regard to possible destruction of blood. Skeletal muscle, in contrast to heart muscle, showed no disease. Sections of the spinal cord and of the trigeminal, spinal and sympathetic ganglions showed no lesions. The spinal nerve roots, the sciatic and femoral nerves and the nerves of the lumbar and of the brachial plexus showed no lesions even in those few cases in which animals were not killed until after two or three weeks.

Lesions of the central nervous system were slight and infrequent as compared with those caused by cyanide, insulin and asphyxia. Rare scattered necrotic neurons were found in the cerebral cortex of the lateral surfaces in a few animals. The only well developed necrosis involved the medial amygdaloid nuclei and the cortical cells of the olfactory tubercle in 2 rats, and these were restricted to very

TABLE 7.—*Protocols of 7 Representative Rats Receiving Fluoracetate*

Rat	Dose and Duration of Treatment	Brain	Heart	Testis	Kidney	Liver
49R28	3 mg. per kilogram twice in 8 hr.	0	2+	2+	1+	Central necrosis 2+
49R29	2 mg. per kilogram daily for 6 days	0	1+	2+	1+	0
49R20	2 mg. per kilogram daily for 6 days	0	1+	2+	0	0
49R43	2 mg. per kilogram 3 times in 4 hr.	(Cortex) 1+	2+	2+	0	0
49R72	2 mg. per kilogram 3 times daily for 2 days	0	3+	(Ovary) 0	0	0
49R87	1.5 mg. per kilogram 4 times in 3 days	0	2+	0	0	0
49R14	1 mg. per kilogram twice daily for 9 days	0	1+	3+	0	0

small foci. Thalamic, brain stem and cerebellar lesions were absent. The substantia nigra, the corpus callosum and the other white matter were spared in all cases. Proliferation of microglia and swelling of vascular endothelium were frequent but slight in the more severely poisoned animals.

Lesions occurred in the hearts of all animals but 1, though in some they were mild. The more severely damaged myocardia showed scattered focal and confluent areas of necrosis of muscle fibers with marked proliferation of mononuclear cells (fig. 9). These cells had oval or round nuclei often with a longitudinal bar of chromatin. They appeared to arise from the interstitial and perivascular connective tissue, as well as from the endothelium of the endocardium and the capillaries of the heart, and became numerous within twenty-four hours. In these cells mitotic figures were abundant. Occasional polymorphonuclear leukocytes appeared in the necrotic lesions, but they were not outstanding. In 1 animal killed four days after poisoning an estimated one third of the ventricle was involved and the wall of the atrium was almost completely diseased, with resultant mural thrombosis and beginning organization. The less severely damaged hearts showed much less necrosis, the foci being made conspicuous by proliferation of the interstitial cells described. From these experiments it could not be shown that the auricles were consistently more severely damaged than the ventricles, although in a few animals they were.[21]

21. Muss, J., and others: J. Biol. Chem. **129**:303, 1939.

Fig. 9.—Fluoracetate poisoning. Ventricular myocardial necrosis in rat 49R43 (table 7). (Hematoxylin and eosin; × 250.)

The testicles of almost all males examined showed varying degrees of necrosis of the germinal cells of the seminiferous tubules, with characteristic confluent masses of several to many dead or dying cells. This necrosis developed rapidly after poisoning, as did that in the heart.

The liver in several instances had focal areas of necrosis in the central zones. None of these was extensive, and in only 1 animal was the process fairly severe. No animals were jaundiced. The kidneys of several animals were the site of tubular necrosis, rarely severe. In most instances of the mild involvement the loops of Henle showed scattered necrotic epithelial cells, but occasionally small foci of necrosis with deposition of amorphous basophilic material suggesting calcification occurred in the proximal convoluted tubules.

In the fluoroacetate series there were several animals on which complete microscopic examinations were not carried out, for two reasons: (1) A few female rats were examined expressly to determine whether the ovaries were damaged, and it was found that they were not: (2) several animals recently dead were included in the fluoracetate series. Although autolysis prevented satisfactory examination of the brains of these rats, it was possible to determine by virtue of the obvious cellular inflammatory response whether necrosis of the heart and some other organs had occurred.

In addition to these experiments 3 rats were studied from the point of view of possible fatty and glycogen changes in the heart and glycogen in the liver. One animal was given 3 mg. of sodium fluoracetate per kilogram (usually a fatal dose) and killed in about three hours, just after the onset of the convulsive episode. Although a few perivascular monocytes had appeared in the myocardium, fatty change or alterations of the glycogen content had not occurred. The glycogen content of the liver was similarly normal. Two additional animals were given 1.5 mg. per kilogram four times over a period of eight hours[22] and then were killed at the end of twenty-four hours. No fat or glycogen changes occurred in the hearts, although in both moderate typical lesions appeared. However, both livers showed moderate depletion of glycogen; in one animal minimal central necrosis had developed while in the other none was present.

Summary of Results.—One may summarize the principal lesions in the experiments (table 8) as follows: cyanide produced lesions in the cerebral cortex, the corpus striatum, the hippocampus and the substantia nigra. It also damaged the corpus callosum and showed a tendency to damage the white matter of the corpus striatum more than did the other agents. The cardiac, hepatic, renal and testicular tissues, the skeletal muscle and tissues of other organs showed no lesions.

Insulin hypoglycemia produced lesions in some respects similar to those caused by cyanide, but there were also differences. The corpus callosum and the substantia nigra were not injured, and the striatal white matter was not conspicuously affected. Destruction of the pyramidal cells of the hippocampus was noted, and, unlike the effect of cyanide, the cells of the fascia dentata were also damaged. In further contrast to the effect of cyanide, the cardiac muscle was injured in a fair number of instances, but the injury was nearly always much milder than that caused by fluoracetate.

22. This dosage was fatal in 4 of 6 other rats.

The animals which were asphyxiated showed lesions in the cerebral cortex, the corpus striatum and the substantia nigra only. A nearly constant finding was minimal cardiac damage, never as severe as in most of the fluoracetate animals. Hepatic, renal and testicular tissues were never involved.

As to the damage done to the brain by fluoroacetate, actual necrosis was uncommon and never extensive. Many animals showed no lesions of the brain at all. Notable was the cardiac damage, sometimes severe enough apparently to cause the death of the animal. A nearly constant finding was necrosis of the germinal epithelium of the testes. Occasionally, hepatic necrosis occurred, although it was usually entirely absent. In the kidneys mild tubular damage was moderately frequent, and in 1 or 2 severely poisoned animals tubular necrosis was possibly extensive enough to have contributed to death. In more than half the animals, however, renal destruction was absent.

TABLE 8.—*Summary of Principal Lesions Caused by Cyanide Poisoning, Insulin Hypoglycemia, Asphyxia and Fluoroacetate Poisoning in Rats*

	Cyanide Poisoning	Insulin Hypoglycemia	Asphyxia in Nitrogen	Fluoroacetate Poisoning
Brain				
Cortex	+	+	+	+
Corpus striatum	+	+	+	..
Corpus callosum	+	..	..	..
Hippocampus	+	+	..	..
Substantia nigra	+	..	+	..
Heart	..	+	+	+
Testis	..	..	..	+
Liver	..	..	..	+
Kidney	..	..	..	+

COMMENT

A simple explanation of the distribution of the lesions produced in these experiments is not possible; undoubtedly a number of mechanisms are responsible. The significance of individual variations in the physiologic and behavior responses made by the animals to the experimental procedures was probably minimized by considering a number of animals. How different acute injury may be from chronic injury is not yet known. The widely held thesis that the vulnerability of a region of the brain is proportional to the degree to which its blood supply falls short of its metabolic demands under stress offers at best only a partial solution. Areas 13, 14 and 51b along the rhinal fissure were frequent sites of injury, and the cortical capillary bed in these areas is poorer than in some others. Nevertheless, there was no consistent pattern that could be attributed to the vascular factor. Furthermore, if the blood supply were the prime factor, the brain, which depends grossly on aerobic oxidation of glucose should show the same pattern of lesions

whether it was deprived of glucose or of oxygen, or whether its carbohydrate catabolism was interrupted at various stages by enzyme inhibitors. This was not the case; the pattern of lesions in the brain showed several consistent differences, and perhaps even more significant, there were marked differences in the distribution of lesions in other viscera.

It is of particular interest to comapre the responses made by the heart and the brain to the four agents used and to correlate these responses with the known metabolism of these tissues and particularly their relative contents of cytochrome oxidase. Cardiac muscle was readily but mildly susceptible to anoxic damage. It was damaged by lack of glucose in many of the animals treated with insulin, and it was severely injured by interruption of acetate metabolism, one of the pathways by which pyruvate may be oxidized. Cyanide never damaged the heart even in severely poisoned animals. In contrast, the brain was severely damaged in cyanide poisoning, asphyxia and hypoglycemia, while escaping severe injury in fluoroacetate poisoning. White matter tended to be hard hit by cyanide.

Heart muscle is believed to utilize glucose and pyruvate[23] as its two principal energy substrates and depends largely on aerobic metabolism. One would expect that any agent interfering with its supply of oxygen and glucose and interrupting certain phases of pyruvate metabolism would cause damage. On the other hand, cardiac muscle is the tissue richest of all in cytochrome oxidase,[24] the enzyme inhibited by cyanide. This rich reserve may be a factor in its notable resistance to the action of cyanide. The gray matter of the brain is fairly rich in cytochrome oxidase and has a high metabolic rate, while white matter is poor in this oxidase (liver and kidney lie between whole brain and heart).[25] Oxygen, glucose and oxidative pathways involving the cytochrome enzyme complex are obviously important to metabolism in both the gray and the white matter of the adult brain, especially in the forebrain. The propensity of white matter to be damaged by cyanide may be closely related to its paucity of cytochrome oxidase, a matter for future consideration in the study of demyelinizing diseases.[26]

These possibilities must be considered tentative at present; other experiments which are planned or in progress using neopyrithiamine (which antagonizes natural thiamine),[27] acetylpyridine (which antago-

23. Mulder and Crandall.[8a] Olson and others.[8b, c, d] Muss and others.[21]

24. Greenstein, J. P.: Biochemistry of Cancer, New York, Academic Press, Inc., 1947, chap. 8, p. 175.

25. Dixon, T. F., and Meyer, A.: Biochem. J. **30**:1577, 1936. Page.[12] Greenstein.[24]

26. Wolff, A., and Kabat, E. A.: J. Neuropath. & Exper. Neurol. **6**:333, 1947. Hurst.[20]

27. Woolley, D. W.: Advances-Enzymol. **6**:129, 1946. Woolley, D. W., and White, A. G. C.: J. Biol. Chem. **149**:285, 1943.

nizes nicotinic acid),[27] certain 8-aminoquinoline derivatives,[9] azide, fluoride, iodoacetate and 2,4-dinitrophenol may yield further pertinent data. Unexplained is the resistance of the substantia nigra to hypoglycemia and its vulnerability to the anoxic forms of injury. It seems unlikely that in 23 animals this observation would rest entirely on chance. Also unexplained is the rather irregular response of the liver and the kidney to fluoroacetate and the extreme vulnerability of the testes to this agent alone. The susceptibility of the testes might depend on a paucity of the fluoroacetate-sensitive enzyme, or metabolic pathways dependent on that enzyme might be of major importance in testicular tissue. The absence of lesions of the brain stem in fluoroacetate poisoning suggests also that either the effects of this compound are not at all analogous to certain aspects of thiamine deficiency, or if there is a similarity, that the rat may be an unfavorable subject for this particular aspect of the problem.

The possibility that side reactions, peculiarities of cell permeability [28] and secondary biochemical effects may be important has not been excluded. There is some evidence that drugs tend to accumulate more in one tissue than in another, but this does not always parallel pharmacologic or frank toxic action. Handler [29] and Kaplan and Greenberg [30] have studied some of the biochemical changes occurring in vivo following the administration of sublethal and lethal doses of cyanide, azide, fluoride and some other inhibitors of carbohydrate metabolism. Cyanide, among others, caused a rise in plasma glucose, lactate and pyruvate over a period of hours. It is conceivable that a rise in these substances might have a protective action on the heart, but they certainly did not help the brain. The blood and tissue biochemical changes of fluoroacetate poisoning are not well known, but in a single rat in these experiments, dead within three hours after receiving 3 mg. per kilogram of fluoracetate, the blood glucose was 100 mg. per hundred cubic centimeters at death. Depletion of the glycogen in the liver has been mentioned (see a foregoing page), but data on pyruvate were not obtained.

Changes in blood vessels,[31] microglia and astrocytes specifically attributable to any one of the agents were not noted in these studies. For the present, qualitative differences of metabolism among these elements remain to be demonstrated.

In view of the rapidity of the onset of symptoms of cyanide poisoning, a direct primary action on the areas affected seems presently to be

28. Broman, T., and Lindberg-Broman, A. M.: Acta physiol. Scandinav. **10**:102, 1945. Vogt, M.: Arch. f. exper. Path. u. Pharmakol. **178**:603, 1935.

29. Handler, P.: J. Biol. Chem. **161**:53, 1945.

30. Kaplan, N. O., and Greenberg, D. M.: J. Biol. Chem. **156**:525, 1944.

31. Rinehart, J. F.; Friedman, M., and Greenberg, L. D.: Arch. Path. **48**:129, 1949.

of major importance, so that the quantitative aspects of the cytochrome oxidase of various tissues must be a factor to be considered seriously. The effects on the heart of anoxia, hypoglycemia and disturbance of pyruvate oxidation show good agreement with the results of in vitro studies and general observation. There seems little reason to doubt that qualitative differences of the metabolic pathways used by various tissues may be powerful factors in determining the differential vulnerability of various organs and regions of the brain.

SUMMARY

The distribution of destructive lesions of the brain and other organs of rats caused by insulin hypoglycemia, cyanide and fluoroacetate poisoning and by asphyxia was studied with a view to determining the mechanisms involved in the production of selective lesions. Cyanide poisoning, insulin hypoglycemia and asphyxia damaged especially the brain but did not produce identical patterns of lesions. Fluoracetate poisoning was particularly destructive to the heart and the testes but caused little or no injury of the brain. Asphyxia and insulin hypoglycemia produced mild damage in the heart, but cyanide poisoning never did. Cyanide, in contrast to the other agents, tended to be selectively destructive to white matter in the brain.

In view of the different patterns of injury produced in the brain by different agents it is not considered that the variable richness of blood supply of different parts of the brain is the only important factor governing the distribution of selective lesions. Rather, other factors, especially the individual metabolic patterns of different brain areas and other tissues, appear to be of prime importance. In this connection the degrees to which cyanide acts on the heart and the white matter may be significant. Heart muscle, of all tissues the richest in cytochrome oxidase, was spared by cyanide, while white matter, with a very low content of the enzyme, was damaged. Further, fluoroacetate, which is believed to inhibit one phase of pyruvate catabolism, was especially deleterious to heart muscle, which is dependent not only on glucose (the prime substrate of the brain) but also on pyruvate.

These concepts are considered tentative at present, pending the results of studies of other enzyme inhibitors now in progress.

EXPERIMENTAL ARGYROSIS

V. Hypertrophy of the Left Ventricle of the Heart in Rats Ingesting Silver Salts

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NEW YORK

THE DEPOSITION of silver in many organs of rats ingesting silver salts for long periods has been previously reported.[1] Special reference was made to the pigmentation of the basement membranes of the glomeruli and renal tubules and of the tissue between the endothelium and the epithelium of such diverse organs as the thyroid gland and the choroid plexuses of the brain and the eye. The purpose of this communication is to present evidence that long-continued feeding of silver is accompanied by hypertrophy of the left ventricle of the heart of the experimental animal.

MATERIAL AND METHODS

Animals Used.—The ancestors of a large majority of the rats used came from Rockland Farms, New City, N. Y., of a strain almost certainly derived by H. C. Sherman, of Columbia University, from that of Osborn Mendel. Seventeen filial generations in all have been bred by mating brothers and sisters. In this way, through eight or nine years, the stock has become increasingly purebred. Each litter was divided as much as possible into a group given silver salts and another given water.

As previously described, over long periods the animals were given silver nitrate or silver chloride kept in suspension by sodium thiosulfate. In the great majority of cases these silver salts were given in 1:1,000 strength instead of drinking water. Most of the animals, including a great majority of those with essentially normal lungs which were subjected to the final statistical analysis described in subsequent pages, were killed in good health. There were relatively large numbers of spontaneous deaths among the rats eliminated from the final study because of advanced pulmonary lesions. The ages at which the animals were killed varied with many factors, including shortage of food during World War II. As much as possible, silver-treated and water-treated littermates were killed together. A few rats survived to 30 months of age.

Determinations of Blood Pressure.—Many attempts have been made in this department to determine the blood pressure of rats from the tails by the use of the method of Williams, Harrison and Grollman[2] without anesthesia. The results

From the Department of Pathology, New York Hospital-Cornell Medical Center.

1. Olcott, C. T.: (*a*) Am. J. Path. **23**:783, 1947; (*b*) **24**:813, 1948.

2. Williams, J. R., Jr.; Harrison, T. R., and Grollman, A.: J. Clin. Investigation **18**:373, 1939.

were not consistent, perhaps in part because of the lack of proper control of the degree of heat to which the animals were exposed. If the heat was inadequate to relax the blood vessels of the tail, an accurate reading was impossible; if it was excessive, the animal was restless, and a false reading resulted.

The aortas of about 25 rats were cannulated under either pentobarbital sodium or ether anesthesia. With both agents the blood pressure readings obtained seemed to be more closely correlated with the depth of anesthesia than with any underlying pathologic or physiologic change.

In 2 rats whose blood pressures were determined in another department of this medical school by Sobin's [3] modification of the method of Williams, Harrison and Grollman but with the use of pentobarbital sodium, the pressures fell from 121 to 92 and from 102 to 75, respectively, between determinations. Both rats died shortly after the second determinations, clearly from the effect of the anesthetic agent.

In 1941 Dr. Ben Freedman, at Mount Sinai Hospital, New York, determined the blood pressure of 6 of my rats by his modification of the method of Williams, Harrison and Grollman without anesthesia. The 3 rats with the highest blood pressure readings had the highest left ventricular weights when killed four to eight months later, but of course this series is much too small to give results of any significance.

The difficulty my associates and I have experienced in obtaining satisfactory measurements of the blood pressure of rats is unfortunately the all too common experience of others. Some authors have described differences of blood pressure in rats of different strains. With different methods of determination, the observed levels of blood pressure of normal rats fall into entirely different ranges. Thus, Woodbury and Hamilton [4] found the blood pressure of anesthetized animals to be 70 to 100 with a mercury manometer and 145 systolic and 105 diastolic with an optical one. They found the level to be 187 systolic and 138 diastolic when an optical manometer was used on the unanesthetized rat. Shuler, Kupperman and Hamilton [5] found that blood pressure was much lower when measured in the tail than when found with an optical manometer. Considering determinations of blood pressure on the tail alone, one notes very discordant normal values in the literature. They vary with the amount of heat used,[6] with the width of the cuff,[5] with anesthesia [7] and with a number of other factors.

Relation of Vascular Hypertension to Weight of Heart in Man and Experimental Animals.—Scriba [8] said that "Hypertrophy of the wall of the left ventricle and increase of the relative weight of the heart may, if the valvular apparatus is

3. Sobin, S. S.: Am. J. Physiol. **146**:179, 1946.

4. Woodbury, R. A., and Hamilton, W. F.: Am. J. Physiol. **119**:663, 1937.

5. Shuler, R. H.; Kupperman, H. S., and Hamilton, W. F.: Am. J. Physiol. **141**:625, 1944.

6. Proskauer, G. G.; Neumann, C., and Graef, I.: Am. J. Physiol. **143**:290, 1945.

7. Sulkin, N. M., and Brizzee, K. R.: Proc. Soc. Exper. Biol. & Med. **66**:125, 1947.

8. Scriba, K.: Virchows Arch. f. path. Anat. **301**:321, 1938.

intact, be taken as evidence of elevation of blood pressure." Other authors[9] have come to an essentially similar conclusion.

Pässler and Heineke[10] and Mark and Geisendörfer[11] found a positive correlation between increased blood pressure and relative hypertrophy of the left ventricle in dogs with "reduced" kidney tissue. Collins[12] constricted the renal arteries of dogs and found hypertension and a relative increase in the heart's weight without any histologic changes of the kidney.

Chanutin and Ferris[13] and Chanutin and Barksdale[14] found that when vascular hypertension was induced in rats by a "reduction" of the kidney tissue, there was a correlated increase in the ratio of the weight of the heart to the surface area, the latter being a function of the weight.

Schroeder[15] found that "in rats the weight of the heart is probably a more reliable index of the presence of the hypertensive state than is one measurement, or two, of blood pressure under anesthesia."

Halpert and Grollman[16] found a positive correlation between the elevation of blood pressure and hypertrophy of the heart, especially of the left ventricle, in rats with "figure of eight" ligatures applied to their kidneys.

The weights of the hearts of many of the rats receiving water in the experiment to be described have been plotted against their body weights with results similar to those of Walter and Addis,[17] who found a straight line relationship between the log of the heart weight and the log of the body weight. Hatai's[18] values (quoted also by Donaldson[19]) are slightly higher in both categories. However, in the present series both values frequently lie much beyond the maximum values of the others quoted, clearly because so many of the rats were kept alive longer than those in the experiments of others. Cromartie[20] found that the weights of the hearts of rats with hypertension were 14 to 144 per cent heavier than those listed by Donaldson.[19]

The question arises as to the validity of using the terminal heart weight in the presence of emaciation, which was, in passing, never extreme in the present series.

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9. Evans, G.: Quart. J. Med. **14**:215, 1921. Fahr, T.: Virchows Arch. f. path. Anat. **239**:41, 1922. Murphy, F. D., and Grill, J.: Arch Int. Med. **46**:74, 1930. Rytand, D. A.: J. Clin. Investigation **17**:391, 1938. Moore, R. A.: A Textbook of Pathology: Pathologic Anatomy in Its Relation to the Causes, Pathogenesis and Clinical Manifestation of Disease, Philadelphia, W. B. Saunders Company, 1944, p. 968.

10. Pässler and Heineke: Verhandl. d. deutsch. path. Gesellsch. **9**:99, 1905.

11. Mark, R. E., and Geisendörfer, H.: Ztschr. f. d. ges. exper. Med. **74**:350, 1930.

12. Collins, D. A.: Am. J. Physiol. **116**:616, 1936.

13. Chanutin, A., and Ferris, E. B., Jr.: Arch. Int. Med. **49**:767, 1932.

14. Chanutin, A., and Barksdale, E. E.: Arch. Int. Med. **52**:739, 1933.

15. Schroeder, H. A.: J. Exper. Med. **75**:513, 1942.

16. Halpert, B., and Grollman, A.: Arch. Path. **43**:559, 1947.

17. Walter, F., and Addis, T.: J. Exper. Med. **69**:467, 1939.

18. Hatai, S.: Am. J. Anat. **15**:87, 1913.

19. Donaldson, H. H.: Rat: Data and Reference Tables for the Albino Rat (Mus Norvegicus Albinus) and the Norway Rat (Mus Norvegicus), American Anatomical Memoir 6, ed. 2, Philadelphia, Wistar Institute of Anatomy and Biology, 1925.

20. Cromartie, W. S.: Am. J. M. Sc. **206**:66, 1943.

This procedure seems abundantly justified by the experiments of Van Liere and Sleeth,[21] who found the percentage loss of the weight of the heart to parallel the loss of body weight in guinea pigs subjected to starvation.

Determination of Relative Weight of Left Ventricle.—There are practical and theoretic objections to using the weight of the entire heart which are obviated by using the weight of the left ventricle. For one thing, there is no clear line of division between the auricles and the large veins or at the origins of the aorta and the pulmonary artery. For another, there are changes in the weight of the right ventricle which are confusing and irrelevant, especially when associated with various degrees of change in the lungs. At any rate, it is felt that the most significant indication of elevated blood pressure is the weight of the left ventricle rather than that of the heart as a whole.

To obtain the weight of the left ventricle, the heart has been fixed over night in Kaiserling solution no. 1[22] (which contains sodium nitrate and sodium acetate) in an attempt to eliminate edema by the use of this strong solution of these salts. I have weighed all the hearts myself on the same scales and with as standard a technic as possible as to the time the tissues were kept on the weight pans, etc. Thirty per cent of the weight of the right ventricle has been deducted from that of the left, after the suggestion of Rytand and Dock,[23] to allow for the portion of the interventricular septum belonging to the right ventricle but weighed with the left. The resulting net weight of the left ventricle (L) is the numerator of the ratio L/W that expresses the relationship between the corrected weight of the left ventricle and the weight of the rat (W). It is usually between 0.3 and 0.6 Gm. for adult rats.

(The technical procedure for separating the ventricles from the auricles is relatively simple. The heart is removed from the rat's body by cutting all the great vessels as close to the heart as possible. Then, with the heart lying on its anterior surface, one blade of a sharp pair of scissors is inserted through the region of the coronary sinus and the incision continued around the heart between the right auricle and the right ventricle [following the right coronary vessels if these can be seen] and continued, always at right angles to the long axis of the heart, to the original incision. By this method, the ventricles are cleanly separated from the auricles and the roots of the aorta and the pulmonary artery. Both ventricles are then opened and sponged out. After being fixed over night in Kaiserling solution 1, the right ventricle is removed from the left by cutting the flange-like edge of the former flush with the interventricular septum.)

The denominator of this ratio, W, is the last weight of the rat, and for adults it is in the neighborhood of 200 to 400 Gm. The ratio is expressed as L in grams per hundred grams of W. Thus, for an animal with an L of 0.563 and a W of 244 the ratio is 0.563 divided by 2.44, or 0.23 per hundred grams of W.

If an animal was found during life or at autopsy to have a tumor or other condition, such as a galactocele, that would make a significant change in body weight, this rat was omitted from statistical consideration. Rats in several other categories were also omitted from consideration, namely, animals on which autopsies could not be made promptly, rats exposed to direct sunlight, rats treated with

21. Van Liere, E. S., and Sleeth, C. K.: Am. J. Physiol. **116**:635, 1936.

22. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938, p. 380.

23. Rytand, D. A., and Dock, W.: Arch. Int. Med. **56**:511, 1935.

2, 3-dimercapto-propanol N. N. R. (BAL, or British anti-Lewisite) in an unsuccessful attempt to remove silver with this substance [24] and rats subjected to renal "reduction."

SUMMARY OF RESULTS

After eliminating the animals mentioned in the foregoing paragraph there are 433 rats whose *L/W* ratios are open to no technical objection.

TABLE 1.—*Average L/W Values at Various Ages: Males*[a]

Age, Months	Normal Lungs		Diseased Lungs	
	Silver	Water	Silver	Water
1	(3) .20 ± .04	(7) .26 ± .02	(2) .21 ± .05	.....
2	(6) .17 ± .01	(2) .17 ± .01	.....	.....
3- 5	(11) .19 ± .02	(8) .15 ± .01	(3) .18 ± .01	(1) .15
6- 8	(9) .14 ± .02	(5) .15 ± .01	(1) .19	(1) .18 ± .02
9-11	(10) .16 ± .02	(9) .15 ± .01	.....	(1) .17 ± .01
12-14	(16) .16 ± .01	(12) .16 ± .01	(7) .18 ± .02	(3) .22 ± .04
15-17	(11) .16 ± .01	(6) .16 ± .01	(3) .20 ± .04	(3) .17 ± .02
18-20	(11) .16 ± .01	(8) .16 ± .02	(7) .18 ± .02	(3) .19 ± .04
21-23	(7) .18 ± .02	(4) .16 ± .02	(5) .18 ± .02	(3) .16 ± .01
24-26	(3) .24 ± .04	.....	(5) .22 ± .04	.....
27-29	(2) .18 ± .02	.....	(2) .20 ± .02	.....
30-32	(1) .18	.....	(1) .26	.....
Unknown	.....	(5) .15 ± .01	.....	.....

[a] The figure in parentheses shows the number of rats in the group.

TABLE 2.—*Average L/W Values at Various Ages: Females*[a]

Age, Months	Normal Lungs		Diseased Lungs	
	Silver	Water	Silver	Water
1	(3) .25 ± .01	(9) .26 ± .18	(3) .29 ± .02	.....
2	(7) .21 ± .05	(3) .16 ± .01	.....	(1) .24
3- 5	(7) .19 ± .02	(11) .18 ± .01	(1) .20	.....
6- 8	(6) .16 ± .02	(1) .17	.....	.....
9-11	(9) .19 ± .01	(13) .18 ± .01	(3) .15 ± 0	(1) .23
12-14	(14) .18 ± .00	(21) .18 ± .01	(3) .21 ± .07	(1) .18
15-17	(21) .20 ± .02	(7) .16 ± .02	(1) .20	(3) .23 ± .02
18-20	(11) .20 ± .02	(11) .19 ± .01	(5) .20 ± .01	(6) .24 ± .03
21-23	(13) .20 ± .01	(3) .20 ± .01	(1) .25	(3) .17 ± 0
24-26	(7) .22 ± .01	(3) .20 ± .02	.....	(1) .21
27-29	.....	(1) .17	(2) .25 ± .02	.....
Unknown	.....	(2) .18 ± .01	.....	.....

[a] The number in parentheses shows the number of rats in the group.

These 433 rats include 131 males given silver salts, 89 males given water, 114 females given silver salts and 99 females given water. The *L/W* values of these rats have been averaged according to age groups, and the

24. Olcott, C. T., and Riker, W. F., Jr.: Science **105**:67. 1937.

averages are listed in tables 1 and 2. The criteria by which normal and diseased lungs were differentiated will be considered in a later section.

Before a significant analysis of the *L/W* values can be made, a further elimination of animals is desirable:

1. It will be seen that among young rats both the water and the silver treated groups have high *L/W* values. These values are especially high among the youngest animals. Accordingly, 109 rats less than 9 months of age will be eliminated from further statistical study. This has the added advantage of removing rats that have received silver for only short periods.

2. Twenty-one male rats more than 24 months old which received silver but for which there were no control rats given water will be removed.

3. Seven rats of unknown age should be removed.

TABLE 3.—*Proportion of Rats Whose L/W Value Was More Than 0.2**

Age, Months	Normal Lungs		Diseased Lungs	
	Silver	Water	Silver	Water
		Males		
9-11	1/10	0/9	...	0/3
12-14	0/16	0/16	2/7	2/3
15-17	1/11	0/8	1/3	0/3
18-20	1/11	0/8	2/7	1/3
21-23	1/7	0/4	1/5	0/3
		Females		
9-11	2/9	2/13	0/3	1/1
12-14	1/14	4/21	1/3	0/1
15-17	2/21	1/7	1/1	2/3
18-20	6/11	2/11	3/3	5/6
21-23	5/13	2/8	1/1	0/2
24-26	1/7	1/3	...	1/1
27-29	....	0/1	2/3	...

* The numerator is the number of rats whose L/W value was more than 0.2; the denominator is the total number of rats in the age group.

The remaining 296 rats are represented in table 3 in such a way that the number of rats whose *L/W* was over 0.2 is placed as the numerator and the total number of rats in the group as the denominator.

Modification of Weight of Left Ventricle Caused by Pulmonary Lesions.—The lungs of 63 (21.2 per cent) of these 296 rats showed consolidation of one lobe or more and were thus considered to have a conspicuous and definite amount of pneumonia. (In one of these rats the pneumonia was less conspicuous, but there was purulent meningitis.) Twenty of these 63 rats died of natural causes, in contrast to only 6 of the 233 rats without significant pulmonary involvement. The results given in the tables conform with the experience of others who have found that bronchiectatic and pneumonic lesions increase with advancing

age.[25] Rats with diseased lungs tend to have higher L/W values than those with good lungs at the various ages. The L/W value tends to be higher for rats receiving silver than for those receiving water, whether or not they have diseased lungs.

Even more clearly shown is the greater variability of the average values of the L/W ratio in rats with diseased than in those with essentially normal lungs. This is apparent when it is noted in tables 1 and 2 that the average of the mean variation for most of the three month periods (the figure following the $\pm$ sign) is a relatively larger part of the average L/W value for that period (the figure before the $\pm$ sign) in rats with diseased lungs than in those with normal lungs. In the former it is often 1:5 or 1:6; in the latter it is usually in the neighborhood of 1:15 or 1:16.

Clear confirmation of the positive correlation between diseased lungs and relative hypertrophy of the left ventricle is given by the use of the "four-fold table."[26] This may be set up, including both silver-treated and water-treated rats, as follows:

a: 28 rats with L/W of 0.2 or more with diseased lungs
b: 35 rats with L/W less than 0.2 with diseased lungs
c: 49 rats with L/W of 0.2 or more with normal lungs
d: 184 rats with L/W less than 0.2 with normal lungs

Using the formula (chi = deviation [X] / standard deviation [6])

$$(\text{chi})^2 = \frac{(ad-bc)^2 \times (a+b+c+d)}{(a+b) \times (c+d) \times (a+c) \times (b+d)}$$

one obtains 3.76 as the value for chi, which is highly significant. To anticipate: This figure is higher than when the weight of the left ventricle is compared in rats given silver and rats given water. In other words, significant pulmonary disease is a more potent factor in causing a high L/W value than is the administration of silver. However, pulmonary changes do not give the uniformly high L/W values found for very young rats.

L/W Ratio in Rats with Normal Lungs.—To return to the tabulation of the L/W ratios and consider only the 233 rats (55 silver-treated and 45 water-treated males, 75 silver-treated and 58 water-treated females) with essentially normal lungs between 9 months of age and the end of the controls (between double lines on tables 1 and 2)—it will be seen that the average L/W value for male rats given water approximates 0.16, while that for females is rarely over 0.19. The L/W value for rats given silver, all ages and both sexes, is equal to or higher than that for rats given water. This higher value is usually due to increased

25. Saxton, J. A.: New York State J. Med. **41**:1095, 1941.

26. Hawley, P. R., in Simmons, J. S., and Gentzkow, C. J.: Laboratory Methods of the United States Army, ed. 5, Philadelphia, Lea & Febiger, 1944, p. 785.

weight of the left ventricle (L), as the body weights of the two groups show no consistent difference. In passing, it has been determined that the higher L/W values are not due to terminal loss of body weight.

In determining the value of chi for silver-treated rats in comparison with their controls, use has been made of the following diagram with the same 233 rats referred to in the foregoing paragraph:

(*a*) silver-treated rats with L/W of 0.2 or more	(*b*) silver-treated rats with L/W less than 0.2
(*c*) water-treated rats with L/W of 0.2 or more	(*d*) water-treated rats with L/W less than 0.2

For the 100 males	6	49	the chi is 2.29.
	0	45	
For the 133 females	31	44	the chi is 2.52.
	12	46	
For both sexes	37	93	the chi is 3.13.
	12	91	

The last-mentioned chi (3.13) is highly significant, as the probability of this figure occurring by accident is in the neighborhood of 1:400.

Relation of Elevation of L/W to Amount of Silver Received.—The 37 rats with L/W over 0.2 received more silver for a longer time and showed more silver on microscopic examination of their glomeruli than did the 93 rats with lower L/W values, as shown by the following scheme:

Of the 37 rats with L/W equal to or above 0.2 ↓		Of 93 rats with L/W less than 0.2 ↓
65%	received 11 Gm. of silver salts or more (there was a tendency for the rats given silver chloride to have a higher L/W value than those given silver nitrate)	42%
81%	received silver for 401 days or more	47%
95%	on microscopic examination showed 3 + or 4 + silver in their glomeruli	71%

L/W in Rats with Reduced Renal Parenchyma.—Two female rats (1 given silver, 1 given water) were subjected to unilateral nephrectomy and killed sixteen and nineteen months later. The values of L/W fitted into those of the series of rats with two kidneys.

Dr. William Dock "reduced" the kidneys of 10 of my rats by severely damaging one kidney with a clamp and removing its fellow a week or so later. The values of L/W when the animals were killed or died months later were: 0.32, 0.29, 0.25, 0.23 and 0.18 for 5 rats receiving silver and 0.29, 0.25, 0.24, 0.23 and 0.16, respectively, for 5 rats given water. These very high values were apparently largely due to an increase in L rather than to a decrease in W.

Possible Adventitious Change in L/W Ratio.—The question arises as to the possibility that the L/W values of rats given silver rose because silver was actually deposited in the heart. There is direct evidence that this is not the case: 1. The granules of silver found in the heart on microscopic examination were scattered, few and small. 2. Nitric acid digestion of the heart of a rat gave an entirely negative result for silver. Comparison of known amounts of silver nitrate indicated that if there was 1 mg. of silver in the entire heart its presence would be revealed by this test for silver. A trace of silver was recovered by digestion of renal and hepatic tissue.

It is of interest that no more vascular lesions were present in the kidneys of animals receiving silver than in those receiving water. There was no suggestion of necrosis or of significant thickening of the arterial walls.

Another possibility of error that must be eliminated is the possibility that the high L/W values might have been due to anemia, the latter caused by the ingestion of silver. In 19 determinations in rats given silver salts the hemoglobin was 14.6 ± 0.8 Gm., while in 12 determinations in rats given water, it was 15.1 ± 1.1 Gm.; i. e., no anemia was found. (The normal value for man in the Hellige colorimeter used is 14.6 Gm. per hundred cubic centimeters of blood.)

URINALYSIS

It is a well known fact that albuminuria is an apparently normal finding in old male rats.[27] When albumin was determined qualitatively with sodium sulfosalicylate in the urine of rats receiving silver and the urine of those receiving water, no clear difference was found.

Dr. Eyup Canat made a number of quantitative determinations of the protein in twenty-four hour urine samples from the rats. The results showed enormous variations. When repeated urinalyses were made there was shown a strong tendency for the proteinuria to increase through the months. Rats given silver had more proteinuria, on the average, than those given water. This may have been partly due to

27. Jackson, H., Jr., and Riggs, M. D.: J. Biol. Chem. **67**:101, 1926. Bell, M. E.: J. Physiol. **79**:191, 1933. Shih, H. E.: Am. J. Physiol. **113**:120, 1935. Saxton, J. A., Jr., and Kimball, G. C.: Arch. Path. **32**:951, 1941.

the greater age of the animals given silver. Males had slightly higher proteinuria than females. The figures may be rendered as follows:

Twenty-two determinations for silver-treated male rats 19 ± 6 months old gave a maximum of 368, a minimum of 1 and an average 83 ± 89 mg. of protein for 24 hours.

Four determinations for water-treated male rats 15± 4 months old gave a maximum of 18, a minimum of 1 and an average of 11 ± 6 mg. of protein per 24 hours.

Nine determinations for silver-treated female rats 14 ± 3 months old gave a maximum of 340, a minimum of 1 and an average of 75 ± 113 mg. per 24 hours.

Three determinations for water-treated female rats 12 ± 1 month old gave a maximum of 14, a minimum of 1 and an average of 9 ± 26 mg. per 24 hours.

Readings higher than any recorded in the foregoing paragraph were given by a water-treated male rat and a silver-treated male rat whose kidneys had been "reduced."

CREATININE

An attempt was made to get some light on the physiology of the kidney by determining the creatinine clearance. Unfortunately, presumably because of faulty collection, there were only negligible amounts of creatinine in the urine. The determinations of blood creatinine were apparently reliable. Three female littermates were killed when 16 months old. Analyses under the direction of Dr. Kendrick Hare, of the department of pediatrics, gave the following results:

2 rats given silver for 455 days:	0.54 mg. per 100 cc. blood
	0.68 mg. per 100 cc. blood
1 rat given water:	0.68 mg. per 100 cc. blood

Thus, in these animals there was no change in the retention of creatinine on the ingestion of silver.

COMMENT

This experiment was started after an examination of the slides from the case of argyria described by Gettler, Rhoads and Weiss [28] showed renal arteriosclerosis. Their patient was a moderately large man, about 70 years old, whose heart weighed 480 Gm. His blood pressure was not recorded. Kino [29] has been the only author to find significant arteriosclerosis in a patient with argyrosis, a man of 77 years. He main-

28. Gettler, A. O.; Rhoads, C. P., and Weiss, S.: Am. J. Path. 3:631, 1927.
29. Kino, F.: Frankfurt. Ztschr. f. Path. 3:398, 1909.

tained that silver pigmentation of the kidneys is primary, arteriosclerosis secondary. Hill and Pillsbury,[30] considering the condition in man, stated that "argyria . . . produces no significant disturbance of the physiology of the affected organs, or of the general health of the patient."

Silver nitrate or silver chloride held in suspension by sodium thiosulfate was given to many rats to see whether the ingestion of silver salts might cause vascular lesions. In a small number of rats the renal arteries of moderate size showed slightly thickened walls, but these changes were no more common in animals given silver than in those given water.

Although there has been no arteriosclerosis or other vascular change that could be attributed to silver, hypertrophy of the left ventricle has apparently been demonstrated in a statistically significant number of the animals treated with silver for long periods. Anemia was not present, and the increase in the weight of the heart was not due to its silver content. The most likely probability is that the ventricular hypertrophy indicated vascular hypertension.

The genesis of this presumed hypertension was not explained. Advanced pigmentation was present in the thyroid gland and in the medulla of the adrenal gland, but no change was found that is known to cause hypertension. The pigment deposited in the choroid membranes of the brain and the eye also seems to be an incidental finding. The most advanced pigmentation was present in the portal vein and the hepatic histiocytes, neither of which have any apparent connection with hypertension, and in the kidney. Changes in the kidney are known, from studies of man and animals, to be closely related to hypertension. Certainly our repetition of the work of many others who studied the influence of "reduced" kidneys has emphasized the likelihood of a renal factor in the hypertension. There may well have been a humoral mechanism associated with the pigmentation of the basement membrane of the renal tubules, but this cannot be proved or disproved. The possibility remains that changes in the structure of the heavily pigmented basement membranes of the glomeruli may have exerted some mechanical influence on the myocardium. It has been shown previously that these membranes were thickened.[1b] No article has been found describing such a change in the experimental work of others. However, the glomerular basement membranes are thickened in many cases of toxemia of pregnancy, and this thickening is accompanied by albuminuria and hypertension. It is submitted as a possibility that these same three

30. Hill, W. R., and Pillsbury, D. M.: Argyria: The Pharmacology of Silver, Baltimore, Williams & Wilkins Company, 1939, p. 132.

changes, thickened basement membrane, albuminuria and vascular hypertension, may have been present as interrelated factors in the animals ingesting silver.

SUMMARY

Hypertrophy of the left ventricle has been found in a statistically significant number of rats ingesting silver salts for long periods. This condition is presumed to indicate that they had vascular hypertension. It is postulated that the hypertension may have been due to the deposition of silver which produced thickening of the basement membranes of the renal glomeruli.

HYPERTROPHIC ILEAC STENOSIS SIMULATING REGIONAL ENTERITIS

Report of a Case with Necropsy

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THE NUMEROUS cases of regional enteritis, reports of which have accumulated in the literature under that title or one of its six common synonyms, have one prominent characteristic in common, namely, a distinct thickening of the wall of one or more segments of the intestine. That feature of the lesion in the present case led to clinical and gross pathologic manifestations similar to those described in many cases of regional enteritis, but the microscopic observations proved this case to be essentially different from those originally described by Crohn and his co-workers.[1]

REPORT OF CASE

A 15 year old white boy entered the Cincinnati General Hospital in May 1944, complaining of abdominal cramps and vomiting. About one year earlier he first noted periodic attacks of dull cramping pains in the upper part of the abdomen, associated with vomiting and diarrhea, and lasting for about two hours. The attacks occurred usually in the morning before breakfast but occasionally several hours after meals. At times, pyrosis necessitated arising at night to regurgitate. Sodium bicarbonate and other medicaments offered transitory relief only. Stools were watery during attacks but in the intervals appeared normal. The attacks would recur frequently for two or three weeks, and the patient would lose between 10 and 15 pounds (4.5 and 7 Kg.) during these periods. This weight was regained during the intervals of well-being when his appetite was hearty and no residual symptoms remained. The patient was an avid coffee drinker, consuming about 10 cups a day, and in warm weather drinking 15 bottles of Pepsi-Cola a day. For a month before entry the pains shifted from the epigastrium to the midabdomen but were not sharply localized.

The patient's father, deceased, had suffered frequent attacks of dyspepsia of severe nature with hematemesis and melena. He also had had rheumatic heart disease and suffered from chronic alcoholism. The mother suffered from asthma. Two of the patient's nine siblings had been operated on in infancy for pyloric obstruction, and a sister died of Hirschsprung's disease at the age of 10 years.

From the Departments of Pathology and Surgery, College of Medicine, University of Cincinnati and the Cincinnati General Hospital.

1. Crohn, B. B.; Ginzberg, L., and Oppenheimer, G. D.: J. A. M. A. **99**: 1323, 1932. Crohn, B. B.: Surg. Gynec. & Obst. **68**: 314, 1939.

Examination revealed a slender but ruddy lad with the following findings: temperature, 97.8 F.; heart, normal; pulse rate, 120 per minute; blood pressure, 90 systolic and 60 diastolic; lungs, clear; respiratory rate, 24 per minute. The abdomen was not distended, tender or spastic. No masses were felt. The remainder of the examination was unrevealing.

The red cells of the blood numbered 4,600,000 per cubic millimeter; the hemoglobin was 14.4 Gm. per hundred cubic centimeters. The urine was normal. An analysis of the gastric content after histamine stimulation failed to reveal free acid, but another made one week later revealed free acid rising to a total of 60 units, the total acid attaining 87 units.

Roentgen studies of the gastrointestinal tract in April and November 1944 showed essentially normal conditions.

The patient was restricted to a Meulengracht diet at home and during the next few months improved considerably, with gain of weight and sense of well-being. Subsequent analyses of gastric content with histamine stimulation continued to show hypochlorhydria, the maximum value of free acid obtained in three additional studies being 20 units. He returned to the clinic in January 1945 because of the first recurrence of symptoms since the preceding summer. Relief was obtained with adjustment of diet and symptomatic measures. He was not seen again until his final entry, three and a half years later.

The patient was readmitted in October 1948, complaining of chills and fever. During the interval he had suffered intermittently from recurrent bouts of abdominal distress and vomiting, phenomena which were intensified since his marriage in May 1948. His bowel movements occasionally became diarrheic during the attacks but were usually costive. He was able to work only a day or two and then was "laid up" with abdominal cramps for two or three days. About a week before admission he contracted a "cold," which was treated symptomatically, and he gradually improved. However, his abdominal pain was so severe that on occasion he writhed about in bed. About 4 a. m. on the day of admission he was awakened with a severe chill, and thereafter became febrile. He reported that an "x-ray of his stomach" made ten weeks earlier showed "an ulcer which could be well in eleven weeks."

The patient was well developed and moderately well nourished, acutely ill and alert. His face was flushed, and his temperature was 104.8 F. by mouth. The skin was moist and slightly mottled, and the lips and acral parts were cyanotic. The conjunctivas were clear and the pupils normal. Except for slight limitation of motion of the left half of the chest, the lungs were clear, with respirations 36 per minute. The heart, although rapid, was unremarkable; the pulse rate was 150 per minute, and the blood pressure was unobtainable. The abdomen was neither distended nor rigid. Peristalsis was normally active. There were slight tenderness and fulness in the right upper quadrant of the abdomen, but no masses or rigidity were felt. The remainder of the examination, including rectal examination, was unrevealing. There was a hydrocele of the left side, said to be of recent origin.

The red cells of the blood numbered 4,200,000, with 13.0 Gm. of hemoglobin. The white blood cell count was 45,000 (10,600 on the following day, with 78 per cent neutrophils). The urine was clear but showed a trace of albumin. The sediment contained an occasional red blood cell and a few white blood cells. The blood urea nitrogen was 56 mg. per hundred cubic centimeters. The carbon dioxide-combining power was 40 volumes per cent. Serum amylase was not increased. A lumbar puncture resulted in a traumatic tap with the spinal fluid under initial pressure of 40 mm. of water.

A roentgenogram of the chest showed essentially normal conditions. Plain and upright roentgenograms of the abdomen revealed enlargement of both liver and spleen but normal kidney outlines. No free air was demonstrated. Several dilated, gas-filled loops of small intestine lay in the midabdomen. These became more pronounced on the following day but were decompressed by Wangensteen suction. A week after entry a roentgenogram of the chest revealed minimal patchy exudate in the left perihilar region. The roentgenogram of the abdomen showed a tube within the descending duodenum, but the dilated loops of small intestine were still present. Only a small amount of air was demonstrated in the descending colon.

With supportive therapy, the blood pressure rose on the day after entry to 90 systolic and 40 diastolic and was thus maintained thereafter. Despite the use of penicillin and sulfonamides, the temperature continued to range between 103 and 105.8 F., the curve being septic in type. On the third hospital day the scleras were icteric, and minute petechial hemorrhages were observed in the skin and the conjunctivas. Abdominal distention supervened and persisted despite Wangensteen suction. Each of seven blood cultures yielded Aerobacter aerogenes. Cultures of stool and urine were negative. The blood urea nitrogen fell to 16 mg. per hundred cubic centimeters. The serum bilirubin was 4.8 mg. direct and 7.4 mg. total; the thymol turbidity was 5.5 units; the thymol flocculation was 1+ and the cephalin flocculation 3+/4+. The usual agglutination studies and a Weil-Felix test gave negative results.

During the second week in the hospital a chest roentgenogram showed a small amount of pleural fluid on the right and a slight increase in the left perihilar infiltrate. The roentgenograms made at the time of the barium sulfate studies in 1944, when reviewed, showed several loops of terminal ileum with sharp, "spikelike" indentations and a slight alteration of the mucosal pattern. Recent plain roentgenograms of the abdomen showed similar changes in the loops of small intestine, which were outlined by gas. Opaque material, presumably mercury from a ruptured Cantor tube, remained "hung up" in the right lower quadrant, probably in the terminal ileum. This persisted for the last ten days of life. Throughout the hospital course, additional abdominal roentgenograms revealed ascites and gaseous distention of the small bowel.

On October 31 a course of aureomycin was instituted, and the temperature began to drop promptly. However, on November 1, secretions accumulated in the upper respiratory passages caused severe impedance, and a tracheotomy was done. Suction of the secretions resulted in marked improvement in his general state. On November 3, following increasing abdominal distention, an abdominal paracentesis removed 1,950 cc. of cloudy straw-colored fluid with high protein content but no bacterial flora. After this procedure, the temperature returned to normal. Abdominal palpation revealed an enlarged, tender spleen and thickened loops of intestine. An ill defined mass was palpated in the right lower quadrant. The fluid reaccumulated, and 720 cc. of bloody fluid was removed on November 8, but the patient's condition did not improve. The temperature again rose in septic fashion, the patient became very weak, and on November 10 the abdomen became rapidly tautly distended. Another paracentesis removed 3,600 cc. of fluid, which resembled blood but did not clot. Death occurred on November 11, the nineteenth hospital day. The clinical diagnoses were: regional enteritis extending to the mesentery or the retroperitoneal tissues; acute pylephlebitis with thrombosis of the portal and splenic veins; gangrene of the small intestine; septicemia, due to Aerobacter aerogenes.

Necropsy.—A necropsy was done six hours after death and was limited to the abdomen. The terminal sequence of pathologic changes which ended in death was quite obvious. A small sinus had formed between the lumen of the ileum and

Fig. 1.—The serosal aspect of the ileum and the mucosal aspect of the cecum. Note the groove in the terminal ileum formed by abnormal folding of the peritoneum.

Fig. 2.—The ileocecal region. Note the thickening of the muscle layers in the terminal ileum.

the mesentery, resulting in a large mesenteric abscess, which in turn had eroded into a major branch of the superior mesenteric vein. The resultant septic thrombophlebitis extended through the superior mesenteric, splenic and portal veins, with pylephlebitic abscess occurring in the liver. The portion of ileum

and jejunum (199 cm.) drained by the completely occluded portion of the mesenteric venous circulation was gangrenous, and there was generalized sanguinofibrinous peritonitis with about 800 cc. of bloody exudate in the peritoneal cavity. The spleen was massively infarcted and weighed 1,425 Gm. Aerobacter aerogenes was recovered in culture from the heart's blood. All of the tissues were slightly icteric.

The most interesting aspect of this case was the long-standing underlying pathologic condition which involved the distal 160 cm. of the small intestine and which caused the ileomesenteric sinus. As viewed in situ the small intestine presented the proverbial "garden hose" appearance so often described in cases of regional

Fig. 3.—The ileum at the junction of the muscle layers. Hematoxylin-eosin stain; × 160.

ileitis. The tremendously thickened coils of small intestine were glued together by fragile strands of fibrin. No fibrous bands, adhesions or congenital malformations were found except in the ileocecal region. The terminal ileum was slightly "bloused" over a groove which encircled the ileocecal junction (fig. 1). Normally the reflections of peritoneum which form the superior and inferior ileocecal fossae do not completely engirdle the terminal ileum. In this case the mesentery of the terminal ileum fanned out into two elliptic folds, which together completely encircled the wall and formed the groove mentioned. The ileum entered the cecum low at the root of the appendix and formed an obtuse angle with the upper part of the cecum. The lumen of the latter was only slightly larger than that of the ileum, and the inferior cecal bulge was very small.

The gangrenous and thickened portions of the small intestine overlapped but were not identical in location. The 199 cm. gangrenous portion extended from 28 cm. above the ileocecal valve to a sharp line of demarcation in the jejunum near the latter's junction with the duodenum. The thickening of the enteric wall began abruptly at the ileocecal junction and extended 160 cm. to fade out gradually in the jejunum. Although the wall was thickest in the terminal ileum, the thickening was fairly uniform throughout the ileum except for a few somewhat thinner streaks which engirdled the lumen where the intestine was folded back on itself. No true "skip" areas were present.

When the intestine was opened, the thickening of the wall was found to be due to a marked increase in the size of the muscle layers, especially the inner circular musculature (fig. 2). Microscopically, hyperplasia of muscle cells was more prominent than hypertrophy, but there was no evidence of recent proliferative

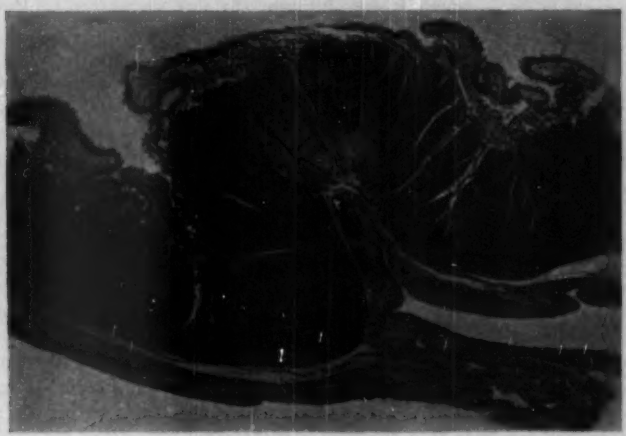

Fig. 4.—A ridge in the ileum with pinching of mesenteric structures. Hematoxylin-eosin stain; × 7.

activity of muscle cells. Fibrosis and inflammatory changes were strikingly minimal, and no sarcoid lesions or ulcerations of the mucosa were found. In places of greatest muscular hypertrophy, the mucosa was atrophic. There were scattered pseudopolypoid projections of mucosa caused by localized edema of the submucosa. Valvulae conniventes were more prominent in the ileum than usual, and the crypts of Lieberkühn contained numerous large, deeply eosinophilic Paneth cells.

In addition to muscular hyperplasia, there was a striking increase in the elements of myenteric plexus (fig. 3). These formed a continuous layer between the circular and longitudinal muscle coats. Many ganglion cells were distorted and vacuolated. Neuronophagia was occasionally seen, but inflammatory changes were minimal except in sections near the gangrenous segment.

At irregular intervals in the ileum, thick rolls of muscle tissue bulged into, encircled and narrowed the lumen. These were U-shaped infoldings of the

muscle coats which, at the site of mesenteric attachment, had apparently dragged lumenward the mesenteric tissues, including nerves and blood vessels, which subsequently became pinched in the narrow muscle-lined crevices thus formed (fig. 4). Vascular occlusion was presumably thus produced, and the resulting local ischemic necrosis probably provided the basis for the formation of the ileomesenteric sinus.

Stomach, duodenum, colon and gallbladder presented low grade chronic inflammation of the mucosa with increased production of mucus. Although there was no muscular hyperplasia in the cecum, the muscle coats of the appendix were definitely thickened, and there was a small mucocele at the tip. The only other important abnormalities were marked emaciation and moderate nutritional cirrhosis of the liver. No pathologic changes were found in the arteries or the nerves of the mesentery, and careful search revealed no sarcoid lesions in the mesenteric lymph nodes. Small globules of mercury were found in the lumen of the ileum and in the ileomesenteric sinus, but apparently were unrelated to the lesion pathogenetically.

COMMENT

In considering the classification of this case one is at once confronted with that convenient diagnostic cubbyhole labeled "regional ileitis" or one of its common synonyms. This case presented, to an impressive degree, a characteristic observed in most of the reported cases of that condition, namely, thickening of a segment of the intestine.

In the literature relating to terminal ileitis the thickening of the enteric wall has been variously attributed to edema, inflammation, fibrosis, lymphatic obstruction or neuromuscular hyperplasia. Among the suggested causes are tuberculosis,[2] schistosomiasis,[3] sarcoidosis,[4] giardia infection,[5] appendicitis,[6] dysentery,[7] food poisoning,[8] psychoneurosis [8] and herpes zoster of Auerbach's plexus.[9] Apparently, any part of the intestine may be involved, from the pylorus to the anal sphincter,[10] and there may be only one or any number of separate segments involved. Several cases which in many respects were similar to the present one have been reported as cases of regional ileitis, especially Schepers' case,[9] in which he thought the ileitis might be caused by herpes zoster because of the degenerative changes in Auerbach's plexus.

In our case the basic lesion was noninflammatory and non-neoplastic. It was essentially hypertrophy and hyperplasia of the smooth musculature of a segment of the bowel. Such changes in smooth muscle are known to occur (*a*) as work hypertrophy when the intestinal wall is

2. Taylor, A. W.: Brit. J. Surg. **33**: 178, 1945.
3. Asencio-Camacho, F.: Gastroenterology **6**: 493, 1946.
4. Hadfield, G.: Lancet **2**: 773, 1939.
5. Barbour, R. F., and Stokes, A. B.: Lancet **1**: 299, 1936.
6. Homans, J., and Hass, G. H.: New England J. Med. **209**: 1315, 1933.
7. Felsen, J.: Ann. Int. Med. **10**: 645, 1936.
8. Bockus, H. L.: J. A. M. A. **127**: 449, 1945.
9. Schepers, G. W. H.: Am. J. Digest. Dis. **12**: 97, 1945.
10. Morehead, R. P.: North Carolina M. J. **8**: 216, 1947.

repeatedly and forcibly contracted against an obstruction, (*b*) as a result of autonomic imbalance when the nerve impulses which cause muscular contraction are not properly balanced by those of inhibition, and (*c*) as congenital abnormalities of muscular development. The interrelationships of factors under (*b*) and (*c*) are not yet clearly defined. For example, hypertrophic pyloric stenosis and megacolon may possibly be caused by congenital abnormalities of the development of the autonomic nervous system, thus leading to autonomic imbalance and muscular hypertrophy.[11]

In the present case there are several possible clues to be considered. It is conceivable that the abnormal reflection of peritoneum around the terminal ileum caused enough constriction to lead to work hypertrophy of the proximal musculature. The fact that the hypertrophy of the ileum stopped abruptly at the ileocecal valve favors this conception. However, in such a condition there usually is little or no hyperplasia. Also, this would not explain the involvement of the appendix, nor why symptoms were so long in developing.

The occurrence of pyloric stenosis and megacolon among the patient's siblings directs attention to familial factors. The fact that when pyloric stenosis occurs in single ovum twins both have it, while in double ovum twins only one of them has it, strongly suggests a genetic causation.[12] Hirschsprung's disease has been reported in five members of one family.[13] Accumulating evidence indicates that both these conditions are caused by some abnormality of muscular growth or innervation during fetal life, and in both conditions there is hypertrophy of smooth musculature. Such changes have been observed in other parts of the gastroenteric tract, including the terminal ileum. In 1926 Fraser[14] reported 3 cases in which hypertrophic ileal obstruction was observed in infants and thought the muscular hypertrophy had resulted from uncontrolled contractive impulses in a segment of bowel which did not have the power of inhibition. In 1945 Guthrie[15] collected a series of cases from the literature and added her own case in which idiopathic muscular hypertrophy occurred in various parts of the gastroenteric tract. In all of these cases the essential features of the lesion were very similar to those in the present case.

Since our patient lived twenty years, we cannot exclude the possibility of postfetal overstimulation of the enteric musculature. When we consider the interrelationships of the sympathetic, parasympathetic

11. Kuntz, A.: Autonomic Nervous System, ed. 3, Philadelphia, Lea & Febiger, 1945.
12. Laubscher, J. H., and Smith, A. M.: Am. J. Dis. Child. **73**: 334, 1947.
13. Muehsam, E.: Am. J. Digest. Dis. **13**: 3, 1946.
14. Fraser, J.: Brit. M. J. **1**: 359, 1926.
15. Guthrie, K. J.: Arch. Dis. Childhood **20**: 176, 1945.

and intrinsic innervations of the enteric muscles and the effects on them of the various endocrine secretions, as well as possible exogenous factors, we have a problem with many variables. However, in the present case the fact that the musculature of the cecum was unaltered is evidence against autonomic imbalance, since there is no sharp separation between the autonomic nerve supply of the terminal ileum and that of the cecum.

Therefore, in the dim light of our present knowledge we have diagnosed this case as hypertrophic ileac stenosis, probably basically congenital in origin but possibly intensified during puberty by other unknown factors affecting the autonomic nervous system.

SUMMARY

A case of marked thickening of the wall of the terminal part of the small intestine is presented, which grossly and clinically resembled regional ileitis. Microscopically, the lesion was found to be intense hypertrophy and hyperplasia of smooth muscle. This was non-neoplastic and noninflammatory in nature and was accompanied by changes in the myenteric nerve plexus. Of interest was the occurrence of congenital pyloric stenosis in two siblings and Hirschsprung's disease in a third. The possible rôles played by heredity, constriction and autonomic imbalance in this case are discussed.

CYST OF THE SPLEEN IN SICKLE CELL ANEMIA

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AND
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CYST of the spleen is uncommon. Several comprehensive articles dealing with its incidence, pathogenesis and symptomatic aspects and with classification of the cases have been published by Fowler,[1] Moynihan [2] and McClure and Altemeier.[3] Parker and Brown [4] described an epidermoid cyst of the spleen in 1948. They reviewed the literature and found reports of 163 cases of nonparasitic cyst of the spleen. We wish to describe a hemorrhagic cyst of the spleen occurring in a patient with sickle cell anemia. So far as we have been able to determine, this is the first reported instance of the simultaneous occurrence of these two conditions.

REPORT OF A CASE

A 21 year old Negro woman was admitted to the Berkeley County Hospital, Moncks Corner, S. C., on the service of Dr. J. N. Walsh, May 24, 1948. Six months before, she had noticed a mass in the left side of the abdomen, which gradually increased in size. The swelling was accompanied by pain. She mentioned no other pertinent symptoms except amenorrhea, which had been noted for the past several months. She was obviously undernourished, and studies of her blood showed sickle cell anemia. A mass was found in the left upper quadrant of the abdomen. The hemoglobin was 45 per cent and the leukocyte count 1,320. The erythrocytes showed widespread sickling, there being 100 per cent deformity in a sickle cell preparation. A preoperative diagnosis of a cystic tumor of the left side of the abdomen was made.

After preoperative preparation, including blood transfusion, a splenic mass was removed on June 13. Spinal anesthesia, supplemented with intravenous injection of thiopental sodium U. S. P., was used. Her recovery following the operation was satisfactory and she was discharged on June 14. By April 1949 she had gained considerable weight and was able to do her housework. She continued to be anemic, however, the hemoglobin being only 45 per cent at that time.

From the Department of Pathology and the Department of Medicine of the Medical College of the State of South Carolina.

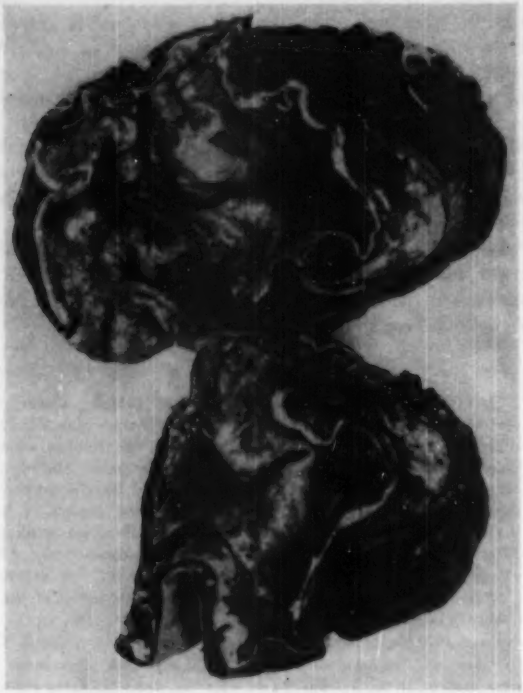
1. Fowler, R. H.: Ann. Surg. **57**:658, 1913; Internat. Abstr. Surg. **70**:213, 1940.

2. Moynihan, B.: Surg., Gynec. & Obst. **40**:778, 1925.

3. McClure, R. D., and Altemeier, W. A.: Ann. Surg. **116**:98, 1942.

4. Parker, E. F., and Brown, A. G.: Surgery **24**:708, 1948.

Pathologic Examination.—Gross Observations: The specimen consisted of a cystic mass measuring 15 by 10 by 13.5 cm. (figure). It was estimated that in the fresh, fully expanded state the specimen was at least one third larger. Its capsule was slate gray with irregular areas of orange-brown discoloration. The surface was slightly irregular owing to elevated firm purple nodular areas. The cavity of the cyst had a gray lining, which was slightly wrinkled and pebble-like in some areas. The lining was coated with a gummy deposit of blood. The



Large cyst with fibrous wall replacing the greater part of the splenic substance.

wall of the cyst measured from 1 to 5 mm. in thickness and on cross section was tough and pearly gray in appearance. The splenic tissue about the periphery of the cyst was firm and the color of mahogany. There were scattered orange-colored deposits and grayish areas here similar to those comprising the wall of the cyst. Blood clot was mixed with the splenic tissue about the cyst, and in some areas it was easily dislodged so as to form other potential false cystic spaces. Several lymph nodes measuring up to 1.5 cm. in diameter were also received.

Microscopic Observations: The splenic tissue about the cyst showed the classic features of sickle cell anemia. There were virtually no normal erythrocytes, the

majority of them existing in long, tapering, oat-shaped or pencil-like forms. All the vascular channels were packed with these sickle cells, and siderofibrosis with formation of typical "Gamna-Gandy nodules" was conspicuous. The cyst wall was composed of organizing fibrous tissue in its inner portion, with a coating of fibrin and fibrinoid material covering its inner portion. Occasional flecks of calcium were present in its wall, and an occasional "Gamna-Gandy nodule" was present in its periphery. Blood pigment was deposited on its surface in some areas. The cyst possessed no organized cellular lining of any description.

The lymph nodes showed conspicuous reticuloendothelial hyperplasia with prominent deposits of hemosiderin.

COMMENT

This cyst is of the so-called false type, i. e., possessing no specific lining. False cysts are encountered much more frequently than those of the true type and the majority of these are the hemorrhagic variety. False cysts have been variously classified; McClure and Altemeier,[5] following Moynihan [2] and Fowler,[1] supply the following classification:

(A) Hemorrhagic
(B) Serous
(C) Inflammatory
  (1) Acute necrosis in infection
  (2) Chronic tuberculosis
(D) Degenerative liquefaction of areas of infarction caused by embolism or arterial thrombosis.

Denneen [5] reported five large hemorrhagic cysts of the spleen. He classified splenic pseudocysts as (1) traumatic, which may arise from a hematoma, and (2) degeneration cysts arising from secondary changes in areas infarcted as a result of arterial degeneration or embolic occlusion of blood vessels with consequent necrosis of the splenic pulp. The cyst described by us probably falls into the latter division. It seems reasonable that many hemorrhagic cysts or even those which are the product of infarction may be subsequently transformed into serous ones through absorption of the blood and necrotic material, with eventual endothelization of the cavity. Splenic infarcts, sometimes of large size, are of frequent occurrence in cases of sickle cell anemia.[6] These infarcts produced by conglutination and stagnation of the erythrocytes or even by actual thrombosis often result in shrinkage of the spleen, so that eventually splenic remnants weighing less than 1 Gm. may occur. This cyst is believed to have resulted from an area of massive infarction, followed by secondary liquefaction, with hemorrhage coming either at the time of the original infarction or as a subsequent complication.

As Diggs [6] pointed out, the spleen of a patient with sickle cell anemia is characterized by a series of progressive changes from congestive

5. Denneen, E. V.: Ann. Surg. **116**:103, 1942.
6. Diggs, L. W.: J. A. M. A. **104**:538, 1935.

enlargement to fibrotic atrophy. A spleen of the size reported here is of distinctly rare occurrence in this disease, and the occurrence of the cyst is even more unusual and to our knowledge this is the only reported instance of a spleen cyst occurring in sickle cell anemia, although the hypothetic mechanism for its pathogenesis is such as to cause one to wonder why it may not be more often observed.

SUMMARY

A large solitary cyst of the spleen of a patient with sickle cell anemia is described. It is believed that the cyst is directly related to the sickle cell anemia and that massive infarction with immediate or subsequent hemorrhage is the background for the evolution of the cyst. This is the one hundred and sixty-fifth case of false cyst of the spleen to be reported and is the first instance in which such a condition has been described as a complication of sickle cell anemia.

LOCALIZED LYMPH NODE SARCOIDOSIS ASSOCIATED WITH CARCINOMA OF THE BILE DUCTS

Report of a Case

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CARCINOMA of the extrahepatic biliary tree is both a rare disease and one which offers considerable diagnostic difficulty. Boeck's sarcoid still eludes the investigator who seeks to shed light on its cause. It is the purpose of this report to present a well documented case in which at autopsy both of these conditions were found to coexist within a well localized area. A review of the literature does not reveal any previously reported cases of sarcoidosis associated with carcinoma. This combination is per se a rarity and the anatomic juxtaposition of both lesions may indicate an etiologic factor contributing to or modifying the development of one or both of them.

REPORT OF CASE

The patient was a 61 year old woman with a chief complaint of jaundice and itching.

She had been previously admitted to another hospital, in December 1947, complaining of jaundice, intense pruritus, light-colored stools and dark urine. These symptoms followed in the wake of a lingering "cold." There had been anorexia, a 10 pound (4.5 Kg.) loss of weight during the previous month, but no use of toxic drugs, no intolerance of fatty foods and no previous episodes of jaundice. The results of the physical examination made at that time were essentially negative except for icterus of the skin and the scleras. The salient laboratory findings were as follows: hemoglobin, 12.7 Gm. in 100 cc.; red blood cell count, 4,320,000; erythrocytic sedimentation rate, up to 38 mm. in 1 hour (Westergren); Wassermann test, negative; blood urea nitrogen, 9.8 mg. in 100 cc.; icterus index, up to 144 units; total serum proteins, 6.6 Gm. in 100 cc. (albumin-globulin ratio, 3.9: 2.7); thymol turbidity, up to 33 units; alkaline phosphatase, up to 8 Bodansky units; blood phosphorus, up to 3.6 mg. in 100 cc.; blood cholesterol, 266 mg. in 100 cc.; blood cholesterol esters, 92.5 mg. in 100 cc. Blood and bile were not present in the stool. Urobilinogen was not present in the urine. Cholecystograms showed a non-filling gallbladder. The Hanger test was negative. The findings suggested obstructive jaundice of undetermined cause. On palpation, a slight resistance was noted in the right upper quadrant of the abdomen without suggestion of a mass.

From the Pathology Laboratory of the Lenox Hill Hospital.

The clinical data concerning this case are presented with the permission of Dr. Carl Eggers.

No operation was done, and the patient was discharged after approximately one and a half months of hospitalization, showing considerable subjective and objective improvement.

After a brief period of well-being, the original symptoms reappeared in addition to some increasing weakness, and the patient was admitted to this hospital on May 2, 1948. She was afebrile. A firm liver edge was palpable at the level of the umbilicus and was associated with a hard mass which was thought to be the gallbladder. The hemoglobin value was 12.4 Gm., the red blood cell count 4,000,000 and the icterus index 64 units. A laparotomy revealed a markedly enlarged, smooth, firm, greenish brown, non-nodular liver. There was a hard mass, about 1.5 cm. in diameter, in the region of the common hepatic duct proximal to the point of entry of the cystic duct. It was felt that this may have been a carcinoma with obstruction of the biliary tract, but the inaccessibility of the lesion made its resection impossible. The mass felt preoperatively was found to be the lower pole of the right kidney which lay in an oblique plane. The gallbladder was contracted and empty. The cystic and common bile ducts were not dilated. An enlarged lymph node lying inferior to the junction of the common and cystic ducts was removed. Histologic examination of the node showed Boeck's sarcoid. The patient improved and was discharged with a diagnosis of obstructive jaundice and Boeck's sarcoid of a perihepatic lymph node.

The symptoms eventually recurred, and the patient was readmitted in November 1948 in no acute distress and without appreciable loss of weight. The liver was still enlarged. The icterus index was 105 units and the serum bilirubin was 20.5 mg. in 100 cc. The hemoglobin was 14.2 Gm.; the red blood cell count was 4,300,000 and the white cell count 8,200. An operation was performed in an effort to establish external biliary drainage. A firm smooth mass was felt again in the region of the porta of a markedly enlarged liver. There was a moderate amount of clear fluid in the peritoneal cavity. Five cubic centimeters of yellowish white mucoid material was aspirated from the gallbladder, and an unsuccessful attempt was made to find a large biliary radicle for drainage. Biopsy of the liver showed biliary cirrhosis. Postoperatively the patient became febrile, her condition grew worse and she died, markedly emaciated, on the forty-fifth hospital day.

Necropsy.—The autopsy was limited to an examination through the surgical incision. The superficial lymph nodes were not palpable. At the lower end of a relatively old, longitudinal incisional scar of the right upper quadrant of the abdomen was a healing sinus which drained a small amount of tenacious, semisolid, yellow exudate. The peritoneal cavity contained approximately 1,000 cc. of clear, thin, dark amber liquid, and all the abdominal organs, particularly the small intestine, were matted together by a dull, ragged, grayish tan exudate which was torn with moderate ease. These adhesions were most marked in the right upper quadrant.

There was an ill defined, irregular, firm, homogeneously gray mass situated in the porta hepatis. This mass measured approximately 3 cm. in greatest diameter at the emergence of the hepatic duct from the liver substance and encircled and constricted the duct at this point. Proximal to the constriction, the main and secondary radicles of the intrahepatic biliary tree were notably dilated and filled with greenish bile. The liver parenchyma was brown, and its structure was discernible but slightly accentuated. Distally the hepatic duct again became wider. The junction of the cystic and the hepatic duct was lost in the mass of firm, gray tissue, and in this region there were several small, greenish cystic structures, measuring up to 0.2 cm. in diameter, which contained a small amount of gray-green mucinous material and which projected into the lumen of the duct.

The common duct was patent and clear throughout, and its wall was of normal thickness. The valves of Heister were normal. The gallbladder was small and of normal shape. The surface was covered with tan fibrous adhesions, and its wall was minimally thickened. The lumen contained a complete cast of dark green-brown, semisolid, putty-like, somewhat stringy material which was slightly adherent to the wall.

There were several firm, enlarged, discrete, gray-green lymph nodes situated to the left of the extrahepatic biliary tree, at the upper margin of the pancreas and between it and the porta hepatis. On cross section the tissue bulged, was moderately firm and rubbery and presented a gray-green homogeneous appearance. The largest measured 4 by 3 by 1 cm. and lay juxtaposed to the bile duct at the point where the hepatic and cystic ducts join. The liver-pancreas-duodenum preparation weighed 1,800 Gm.

The gastrointestinal tract was essentially normal except for several scattered ill defined small subserosal nodules, measuring up to 0.7 cm. in diameter, situated over the small and large intestines. These nodules were sectioned with slightly increased resistance, and the sectioned surface was firm and gray with ill defined softer yellowish cores in the larger nodules. The spleen weighed 300 Gm. but was normal in shape and appearance and somewhat friable. The genital organs were grossly normal. The kidneys had a few superficial pitted scars over which the capsule was slightly adherent, and at the upper pole of the right kidney was a well demarcated, slightly depressed yellow nodule measuring 0.5 cm.

The chest organs were palpated and visualized, and no gross abnormalities were found.

Microscopic Examination.—The gray tissue described in the gross was composed of a large mass of compact fibrous tissue in which were embedded a moderate number of tubules and cords of tumor cells resembling the lining cells of bile ducts. The tumor cells had pleomorphic, hyperchromatic nuclei of moderate size and a granular, slightly basophilic cytoplasm. No mitoses were noted. The tumor cells were rarely single, and an occasional one had a signet ring configuration. The amount of fibrous tissue predominated over the amount of tumor glands, and there was a moderate lymphocytic reaction. In the region of the stricture the dense fibrous tissue was partially hyalinized. Within this area, around and within the nerves, around the blood vessels and in the wall of the hepatic duct were clumps and follicles of tumor cells, some of which were dilated even cystically. This was noted particularly in a section taken through the small cysts described in the gross near the junction of the cystic and hepatic ducts. These cysts were lined by simple tall-columnar epithelium with dark flattened basal nuclei and pale cytoplasm; the lumens were filled with eosinophilic material and desquamated cellular debris. Some of these cysts were dilated; others were small, and their prominent lining showed papillary infolding into the lumen.

The walls of the bile ducts were markedly widened by fibrous tissue. Within the liver, the structure was markedly altered by an irregular network of anastomosing strands of fibrous tissue which apparently originated in the portal areas and within which there was marked bile duct proliferation out of proportion to the amount of fibrosis. This bile duct proliferation and fibrosis were most marked in the vicinity of the porta hepatis and decreased toward the periphery of the liver. In these areas of fibrosis there were a moderate amount of phagocytosed bile pigment and a slight diffuse collection of lymphocytes and a few neutrophilic polymorphonuclear leukocytes. The liver cord cells around the periphery of the fibrous strands were slightly atrophic and dissociated and contained a small amount of bile pigment granules. A few bile ducts were filled with bile casts.

The lymph nodes at the upper margin of the head of the pancreas and in the porta hepatis revealed a structural pattern which was largely obscured by a diffuse seeding with many small granulomas of fairly uniform size. They were usually discrete but not infrequently slightly confluent. The granulomas were composed of epithelioid cells and were slightly infiltrated by lymphocytes and an occasional multinucleated giant cell of the Langhans type. Many of these nodules were undergoing centripetal hyaline fibrosis and many were completely reduced to a hyaline mass. Some of the lymph nodes, particularly those near the porta were criss-crossed by a network of hyaline bands, and only a few granulomatous lesions were present. One node, also near the porta hepatis, contained an area of tumor

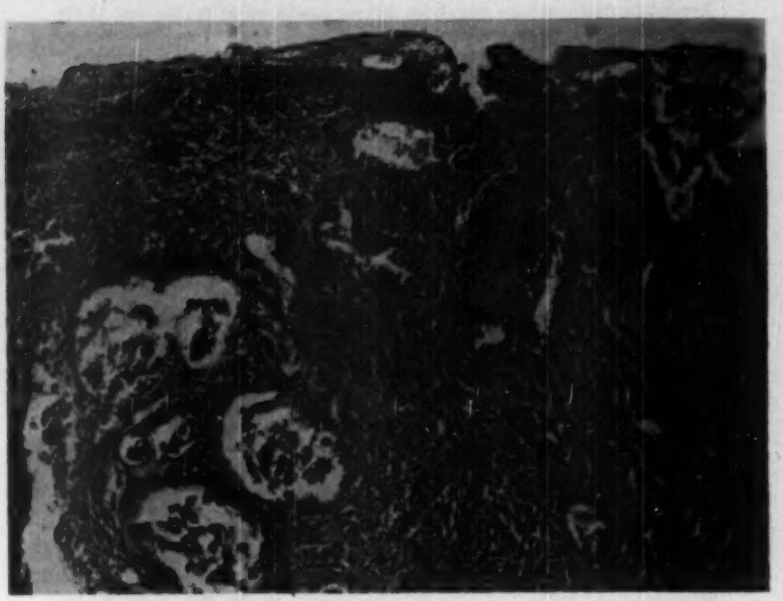

Fig. 1.—Wall of the hepatic duct at the site of stricture. The wall is composed of compact fibrous tissue containing a few tumor glands. The epithelial lining is absent, and there is a superficial zone of necrosis and slight infiltration of lymphoid cells.

tissue. The sinuses of the lymph nodes bore many large mononuclear cells, some of which were laden with brown, non-iron-staining pigment.

The gallbladder wall was moderately widened with fibrous tissue and was slightly hemorrhagic. It was slightly infiltrated with lymphocytes and a few multinucleated giant cells, and contained a moderate amount of diffusely scattered intracellular brown pigment.

The serosal nodules present in the intestine were composed of nests and alveoli of tumor cells similar to those around the hepatic duct and porta hepatis. In one section the tumor penetrated through the muscularis and into the submucosa.

The peritoneal surfaces showed a slight fibroblastic reaction, a slight infiltration of chronic inflammatory cells and a few polymorphonuclear leukocytes, and an occasional layering with organizing fibrin.

In one ovary was a small congerie of cystic spaces lined by tumor cells similar to those described for the main tumor. The spleen showed slight diffuse fibrosis of the red pulp and slight atrophy of the malpighian corpuscles. A moderate number of pancreatic acini were slightly dilated and filled with eosinophilic granular material, and there was a slight lymphocytic infiltration of the pancreatic interstitium. The renal nodule was composed of whorls of mature fibrocytes which slightly compressed the surrounding tissue. No other salient changes were noted.

Fig. 2.—Lymph node from the porta hepatis showing Boeck's sarcoid.

Anatomic Diagnoses.—Adenocarcinoma of the bile ducts with metastases in the small and large intestines and ovary; Boeck's sarcoid of the hepatic and pancreatic lymph nodes; fibrous stricture of the hepatic duct; dilatation of the intrahepatic biliary ducts; biliary cirrhosis; chronic cholecystitis; obstructive jaundice; subacute peritonitis; fibroma of left kidney; fibrosis of the spleen.

COMMENT

This would have been nothing more than a fairly typical, though rare, case of carcinoma of the bile ducts had it not been for the fact that it was also associated with Boeck's sarcoid. This combination both tended to confuse the definitive cause of the obstructive jaundice during

life and raised the question as to whether there is any causal relationship between the two diseases. The latter question is rendered particularly more cogent since both lesions were largely limited to the region of the porta hepatis.

A pathologic diagnosis of adenocarcinoma of the bile ducts could not be made prior to death because of the inaccessibility of the site and because the few metastases discovered at autopsy were either not yet present or were small enough to have been overlooked at laparotomy. Bockus [1] stated that some cases designated as instances of benign stricture of the extrahepatic bile ducts may have been actually instances of carcinoma. This difficulty is brought about by the fact that this type of tumor, particularly the nodular form, produces a marked degree of scarring.[2]

The answer to the question whether there is a causal relationship between the two conditions in this case is admittedly an elusive one. They do appear to meet on some common ground in that both conditions tend toward fibrosis and that both localize in a particular anatomic region in this case.

The apparent focusing of the expression of these two different lesions onto the same area may be purely fortuitous, though this does not seem likely. On the other hand, it may conceivably have been due either to an expression of "regional weakness" or "terrain sarcoidique" preexisting before the occurrence of both conditions, or to a local propensity induced by whichever of the two diseases occurred first for the other.

The fact that both carcinoma of the bile ducts and Boeck's sarcoid are closely associated with intense fibrosis is a more reliable thread of common ground. This characteristic is well established and was present full blown in this case. If fibrosis and scarring are accepted as being expressions of host resistance, the question arises whether this is due to a factor or factors common to both of these diseases. It may be that Boeck's sarcoid is a type of tissue response indicating resistance of the body: in this case, resistance to carcinoma.

No definite answer to these questions can be derived from the study of a single case nor can generalizations be made. However, facets are presented which deserve consideration.

SUMMARY

A case is presented and discussed in which the simultaneous presence of carcinoma of the bile ducts and Boeck's sarcoid in a localized area was proved at necropsy.

1. Bockus, H. L.: Gastroenterology, Philadelphia, W. B. Saunders Company, 1946, vol. 3, p. 642.

2. Ewing, J. E.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1940, p. 759.

EFFECT OF CHOLESTEROL FEEDING IN HAMSTERS

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ATHEROMATOSIS has been produced in rabbits, chickens and dogs by feeding of cholesterol. The investigators have inferred that the production and degree of atheromatosis showed a certain correlation with the blood cholesterol level. This level, in turn, depended largely on the amount of cholesterol administered and the length of time the animals were fed cholesterol.

Each of the aforementioned animal species offers some advantages and has some disadvantages. In the rabbit, one has to produce high grade hypercholesteremia before vascular lesions develop. This in itself makes the mechanism of production dissimilar from the process in man, in whom the blood cholesterol level is not significantly increased, prior to or during the development of atheromatosis. Furthermore, in old animals, spontaneous vascular lesions occur, so that with lengthy feeding schedules one is unable to evaluate properly the effect of cholesterol. In chickens spontaneous atheromatosis develops to a high degree. Therefore only very young birds can be used for the experiments. Even with young birds one has to gage atheromatosis by comparing the degree of alterations in experimental birds as compared with control birds. To produce atheromatous alterations in dogs it is necessary to administer thiouracil simultaneously with cholesterol. This, again, makes the mechanism dissimilar to that in man.

A large variety of animals has been experimented with to find a more suitable species for the study of atheromatosis. Our study was undertaken to determine the possibility of producing atheromatosis in the Golden Syrian hamster. Only sporadic references have been made relative to experimental atheromatosis in hamsters. Altschul [1] stated that "in addition to rabbits and guinea pigs, golden hamsters also are susceptible to cholesterol arteriosclerosis." Deuel and Marx mentioned "that dietary cholesterol can induce aortic lesions in guinea pigs, rats and hamsters (as well as in rabbits)." [2a] In hamsters subsisting on a diet containing 1 per cent of cholesterol in cottonseed oil plus 0.25 per cent of sodium glycocholate,[2b] only minimal sclerosis developed.

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1. Altschul, R.: Arch. Path. **42**:277, 1946; Am. Heart J. **38**:480, 1948.

2. (*a*) Deuel, H. J., Jr.: Life Insurance Research Fund, 3rd Annual Report, 1947, p. 21. (*b*) Marx, W.: Personal communication to Pollak.[3]

MATERIALS AND METHODS

Batches of hamsters of the same sex (male), age (3 months) and weight (90 to 100 Gm.) were obtained from the same hamstery. Two problems arose:

1. How to feed cholesterol to hamsters.

2. How to obtain blood from the hamsters for determinations of cholesterol.

Incorporating cholesterol into the feed, as is sometimes done for other animals, was impracticable for the hamster because this animal stores food in its buccal pouches. There would be no assurance as to the hamster's consumption of the cholesterol. Tube feeding of hamsters was found very difficult. The method of feeding cholesterol in capsules, used by Pollak [3] for rabbits, was adopted. Each gelatin capsule no. 00000 was filled with 50 mg. of powdered cholesterol (Armour Laboratories). Whereas rabbits swallow even larger capsules readily, hamsters offer resistance. This difficulty was eliminated by anesthetizing the hamsters with ether. The capsule was placed in the hypopharynx by means of a forceps. The animal was allowed to awaken from the mild anesthesia and was then observed until the capsule was swallowed. Each animal was fed 50 mg. of cholesterol daily for various lengths of time.

TABLE 1.—*Number of Hamsters, Number of Days Animals Were Fed Cholesterol, and Amount of Cholesterol Fed*

Hamsters	Days of Experiment	Amount of Cholesterol Fed, Gm.
17	30—80	1.0
15	30	1.5
10	60	3.0
29	90	4.5
12	130	6.5
7	30	0
5	60	0
13	90	0
6	130	0

To obtain blood from hamsters, punctures of the jugular vein and of the femoral vein were attempted. These efforts were unsuccessful. Only by cardiac puncture could sufficient blood to produce 0.5 cc. of serum be obtained. This amount does not suffice to assay the proportions between free and esterified cholesterol. Total blood cholesterol was determined, only. The Sperry-Schoenheimer method was employed. A large number of animals died as a result of cardiac puncture. Animals accidentally killed before the lapse of thirty feeding days were considered separately. Cardiac puncture could not be repeated. Therefore, blood cholesterol was determined but twice—at the onset of the experiment and after death.

When an animal was killed, the liver was weighed and its cholesterol content established. Because of the small amount of material left after procurement of tissues for histologic study, the cholesterol was determined per wet weight rather than per dry weight. The organs were extracted with a mixture of equal parts of acetone and of 95 per cent ethyl alcohol, and the Sperry-Schoenheimer method was used.

Seventy-seven hamsters were fed cholesterol. Seventeen hamsters died before the thirtieth day of observation as a result of cardiac puncture. Thirty control hamsters were killed at intervals along with the cholesterol-fed animals. All hamsters were maintained on a diet consisting of lettuce, pablum*, purina* pellets and water.

3. Pollak, O. J.: Arch. Path. **37**:337, 1944.

RESULTS

Three different batches of hamsters (A, B and C,) showed variation as to normal levels of blood cholesterol, but within each batch of animals the normal levels were fairly uniform. Series B and C were grouped together because of the close similarity of their blood cholesterol levels. The initial blood cholesterol values are charted in table 2 as are the values obtained after cholesterol feeding.

All three groups of hamsters, A, B and C, had an average normal liver weight of 2.36 Gm. There was, however, considerable variation in liver weights. See table 3.

The amount of liver cholesterol was doubled after twenty days of cholesterol administration and it was four times the original level after one hundred and thirty days of feeding. The average liver weight increased on cholesterol feeding.

TABLE 2.—*Blood Cholesterol of Hamsters Before and During Cholesterol Feeding*

Days of Cholesterol Feeding		0		20	30	60-120
Hamster series A	Minimum cholesterol value*.....	50		122	158	0
	Average cholesterol value.....	60		152	168	0
	Maximum cholesterol value.....	117		185	180	0
Hamster series B and C	Minimum cholesterol value.....	116	123	0	0	103
	Average cholesterol value.....	142	143	0	0	177
	Maximum cholesterol value.....	166	157	0	0	396

* Mg. of cholesterol in 100 cc. of blood.

TABLE 3.—*Weight of Hamster Liver and Cholesterol Content of Liver Before and After Cholesterol Feeding: Maximum, Average and Minimum Values*

Weight of Liver, Gm.		Cholesterol Content of Liver, Mg. in 100 Gm. of Wet Liver	
At Onset	After Feeding	Onset and Controls	After Feeding
1.50	2.00	488	1,700
2.36	2.78	600	2,100
3.70	4.13	1,800	2,580

Comparison of the weight was impossible because of the great fluctuation of the normal weight of the liver.

POSTMORTEM OBSERVATIONS

Sections were taken from all organs of all animals. Microscopic examination revealed steatosis of the liver in all cholesterol-fed hamsters. The degrees of steatosis were comparable to the results of chemical analyses of the livers. The adrenal glands of these animals presented steatosis. Testicular atrophy, the degree of which appeared to be parallel to the degree of steatosis, was observed.

Isolated foam cells and groups of two to three foam cells were observed in the subintimal layer of the ascending aorta in but 4 of the 77 cholesterol-fed hamsters. The blood cholesterol values and feeding periods of these 4 animals were: 112 mg. in 100 cc. after ninety days, 292 mg. in 100 cc. after one hundred and thirty days, 238 mg. in 100 cc. after ninety days and 342 mg. in 100 cc. after sixty days. None of the 60 hamsters had any appreciable degree of atheromatosis.

COMMENT

The amount of cholesterol fed to hamsters was calculated so as to imitate the experimental conditions which commonly produced cholesterol atheromatosis in the majority of rabbits so treated. The length of the feeding periods was similarly calculated. The discouraging results of our experiment were to be expected on the basis of an analysis of the literature concerning the atheromatosis produced in hamsters, which compared the results with those obtained in rats and guinea pigs. The last two animal species are commonly held unsuited for experimental production of cholesterol atheromatosis. The hamster might well be added to those gnawers.

SUMMARY AND CONCLUSIONS

Hamsters were fed cholesterol for periods of from thirty to one hundred and thirty days under proper control. Blood cholesterol elevation, steatosis of the liver and the adrenal glands and testicular atrophy were observed in hamsters fed cholesterol. In no animal did any appreciable degree of atheromatosis develop in the course of the experiment. The Gold Syrian hamster seems ill suited for the experimental attempt to produce atheromatosis by the feeding of cholesterol.

TRYPTOPHAN DEFICIENCY IN THE RAT

Histologic Changes Induced by Forced Feeding of an Acid-Hydrolyzed Casein Diet

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AND
HARRY SPECTOR
CHICAGO

IN AN EARLIER study (Spector [1]) of the effects of tryptophan deficiency in the rat, a few sections were made from the liver of one of the experimental animals. The sections showed a heavy infiltration of fat. It was decided, therefore, to investigate this condition more thoroughly and also to study the general histologic picture of tryptophan deficiency in the rat, since little is known concerning it. The detailed findings are presented herewith.

MATERIALS AND METHODS

Young male and female rats of the Sprague-Dawley strain were fed Purina® laboratory chow until 6 weeks of age to equalize their weights. At this time they were transferred to an experimental diet [2] containing acid-hydrolyzed casein and ample mineral and vitamin supplements, similar to that by which tryptophan deficiency had been previously established (Spector [1]). The deficient diet was supplemented with 0.2 per cent l-tryptophan for the controls. Both controls and experimental animals were forced-fed with equal amounts three times daily, the technic described by Spector [1] being used. The food intake was gradually increased from 7.5 to 12 Gm. daily over the period of the experiment. The forced feeding technic was adopted in an attempt to avoid the complications which frequently arise as a result of the inanition which almost always occurs when experimental animals are given diets deficient in an essential amino acid.

From the Department of Zoology (Mr. Adamstone) and the Division of Animal Nutrition (Mr. Spector), University of Illinois.

1. Spector, H.: J. Biol. Chem. 173:659, 1948.
2. The composition of the tryptophan-deficient ration was as follows:

	Per Cent
Acid-hydrolized casein	20.0
Casein (Labco, vitamin-free)	2.0
Salts 446 (Illinois) (Spector [1])	5.0
Corn oil	3.0
dl-methionine	0.6
Sucrose	69.4

The tryptophan content of this ration was 0.024 per cent, all coming from the Labco casein.

Originally it was planned to kill 1 male and 1 female from the group fed the deficient diet on alternate days over a period of three weeks. In addition, 1 male and 1 female control were to be killed on the seventh, fourteenth and twenty-first days. This proved impossible because of unforeseen circumstances, but the schedule was adhered to as closely as possible. The rats were killed by decapitation, and histologic samples were secured as follows:

1. A series of samples of nearly all tissues were removed from each animal and fixed in Bouin's fluid. At the same time the brain was removed and fixed in 95 per cent alcohol. These tissues were later embedded in paraffin and sectioned at 10 microns, routine procedures being used. All tissues were stained with Harris' hematoxylin and eosin except the brain sections, which were stained with cresyl violet.

2. In addition to the aforementioned material, a separate piece of liver tissue was removed and immediately frozen on the freezing microtome with carbon dioxide. This tissue was sectioned at once, and a number of slides were prepared by modified frozen section technics (Adamstone and Taylor [3]). The sections were fixed and stained in a variety of ways, as shown in the table. All of these preparations were made with the extreme rapidity and facility which this

Preparation of Tissues

Fixative	Stain	Purpose
F. A. A. (Lavdovsky)	Harris hematoxylin, orange G	General structure
Cadmium formol*	Sudan IV and Harris hematoxylin or light green SF.	Fat distribution
Altmann's osmic bichromate	No stain	Fat distribution
Altmann's osmic bichromate	Altmann's picric acid-fuchsin	Mitochondria
F. A. A. or 5% hydrochloric acid	Feulgen	Nucleic acid

* Baker, J. R.: Quart. J. Micr. Sc. 85:1 (pt. 1), 1944.

method makes possible, and the cells showed neither distortion due to shrinkage nor loss of included materials, such as fat.

It is pertinent to point out that all rats were killed about 9 a. m., at least fourteen hours after the last feeding. Therefore, the cycle of hepatic fat turnover should be at approximately the same stage in relation to feeding time in all cases. Hence, sections of the liver tissues, obtained from the different animals should be comparable with one another.

There were four groups of experimental animals receiving the diets indicated in the following outline. (The number of animals available for histologic study in each case is shown in parentheses.)

(*a*) Stock diet group (2).

(*b*) Control diet group (5): These were fed the deficient diet with 0.2 per cent l-tryptophan supplement.

(*c*) Tryptophan-deficient diet group (12).

(*d*) Curative diet group (3): These were fed the tryptophan-deficient diet for ten days, then the control diet for seven days, to show the response to tryptophan therapy.

As shown, tissues were available from 22 animals taken from all groups for histologic study, and the general histologic picture of the effects of tryptophan

3. Adamstone, F. B., and Taylor, A. B.: (*a*) Anat. Rec. (supp.) **99**:639, 1947; (*b*) Stain Technol. **23**:3, 1948.

deficiency as presented by them appears to be sufficiently consistent and clearcut to warrant the conclusions presented in this article.

HISTOLOGIC OBSERVATIONS IN GENERAL

A great majority of the tissues of these rats showed no striking abnormality. This was true of the following structures: brain, lung, kidney, spleen, pancreas, thyroid gland, thymus, adrenal gland, skin, ovary, oviduct and vagina. By contrast with the structures just listed, other structures presented lesions of a more or less severe nature as described in the following sections.

LIVER

Fat Distribution.—Since the preliminary studies of the liver had suggested a heavy infiltration of fat, this condition was studied in preparations of frozen sections in which the fat was stained with sudan IV or with osmic acid.

1. Stock Animals: In these 2 rats the amount of fat present in the liver was quite small and was distributed in a similar and characteristic manner in both. In individual lobules of the liver (fig. 1) the fat was largely confined to the peripheral zone, with the greatest concentration in the periportal areas. The fat droplets were generally of moderate size. Small amounts of fat were also present in the cells of the radiating hepatic cords near the central vein. The droplets, however, were small, and the whole area was relatively free of fat.

2. Control Animals: As indicated, tissues were available from 5 animals in the control group. These were killed so that 1 was available on the seventh, 1 on the ninth and 3 on the twenty-third day of the experiment. Liver tissues of these animals are illustrated by figures 4 and 7. While chemical analysis (which will be presented elsewhere) has shown considerable variation in total fat content in these livers, all of them presented a similar histologic picture of the distribution of the fat.

In these rats the region immediately surrounding the central vein was again relatively free of fat, although some small droplets were present. Most of the fat was confined to the periphery of the lobule, with the greatest concentration in the periportal areas, and the fat droplets were of moderate size. Thus the distribution of fat was essentially similar to that seen in the livers of the stock animals described.

3. Tryptophan-Deficient Animals: Tissues from 12 rats were available for study in this group. These were obtained from animals killed at intervals from the second day until the end of the experiment. The general change in the distribution of fat in the liver over this period is illustrated by figures 2, 3, 5, 6 and 8. These figures show that there was a gradual increase in the amount of fat in the liver during the course

of the experiment, although considerable individual variation occurred. Thus, the histologic picture of the distribution of fat shows a gradual change from a condition similar to that seen in the stock or the control animals to an entirely different pattern produced when tryptophan deficiency had become more severe. In livers secured from animals during the first week the fat was still most abundantly distributed in the peripheral zones of the lobules and in the periportal areas. In these areas individual fat droplets were still of moderate size on the second and fifth days, but much larger drops appeared in the seventh day liver. The region surrounding the central vein was still relatively free of fat although some small droplets had begun to appear.

From the ninth until the fifteenth day the histologic picture showed a decided and continual increase in the amount of fat in the lobules with an increasing encroachment on the area surrounding the central vein. Ultimately the entire liver was almost completely infiltrated with fat. The droplets in the individual cells had also become progressively larger, owing to coalescence of smaller drops (compare figs. 10 and 11). This was accompanied by gradual isolation of the nucleus, so that it became completely surrounded by fat (fig. 11). In some cases the drops of fat became so large that the cell walls were ruptured and the neighboring drops of fat coalesced to form large, irregular globules.

4. Curative Diet Animals: Three animals which had received the tryptophan-deficient diet for ten days were given dietary supplements of l-tryptophan for a period of seven days before being put to death. The livers of all of these showéd a great reduction in the amount of extracted fat as well as a return to a nearly normal histologic picture of fat distribution (fig. 9). In the lobules, the area around the central vein was again almost devoid of fat, but there was a fairly heavy concentration in the periportal regions. This fat, moreover, consisted of large drops, attesting to the probability that a heavy infiltration of fat had developed before curative treatment was begun.

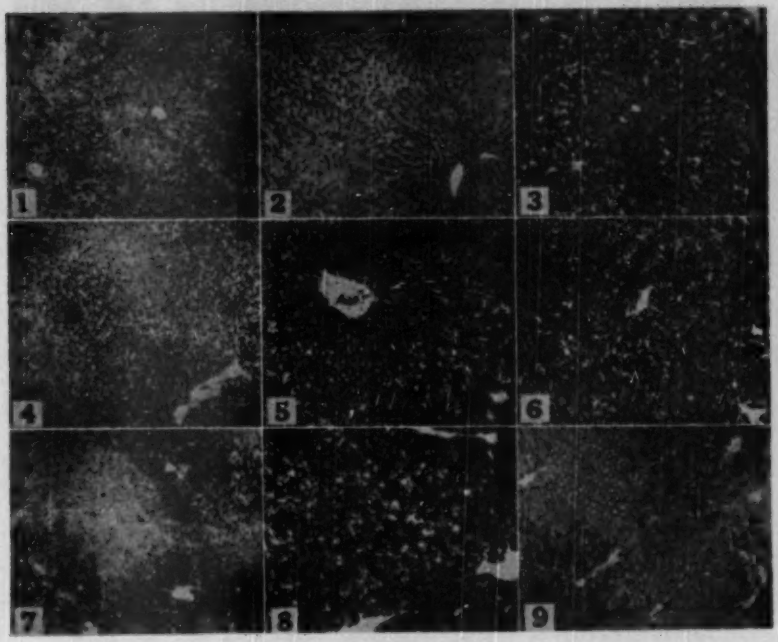
The results of this study have shown that in the livers of tryptophan-deficient rats there is a rapid increase in fat deposit after the first week, so that the entire organ becomes heavily infiltrated with it. The character of the fat droplets changes during this period, so that at the time of greatest accumulation the cells contain huge drops which almost completely surround the nucleus and displace the cytoplasm.

It is apparent, however, that the accumulation of fat produces no serious permanent injury demonstrable by routine histologic technics, provided curative treatment is begun early enough.

Nucleoli.—In addition to the change in the distribution of fat which accompanies tryptophan deficiency, the liver cells showed striking alterations in the structure of the nuclei. In the rat the nucleus of the normal cell (fig. 12) usually contains from two to four nucleoli of moderate

size. In the tryptophan-deficient rats, by contrast, after only two days of the experimental diet many of the nuclei had only a single huge nucleolus (fig. 13). It is probable that this was formed by fusion of the smaller nucleoli, although proof that it was could not be obtained. It is interesting to note that in the nuclei of animals given curative treatment there were indications of a return of multiple nucleoli.

Mitochondria.—Toward the end of this work we had succeeded in applying Altmann's mitochondrial stain to the frozen liver sections, so that study of the mitochondria became possible. With this staining



Figs. 1 to 9.—Distribution of fat in the liver. Fat is represented by black globules ranging in size from minute droplets to large globules. × 26.

Stock animal: Figure 1. Fat is concentrated at the periphery of the lobule. The fat droplets are small.

Control animals: Figures 4 (9) and 7 (23) (days on experiment are indicated in parentheses). The distribution of fat is similar to that in the stock animal.

Tryptophan-deficient animals: Figures 2 (2), 3 (3), 5 (14), 6 (14, mature animal) and 8 (15). These show fat distribution in the livers of animals fed the tryptophan-deficient diet for the number of days indicated in parentheses. In figure 2 the distribution of fat is similar to that in control animals. In figures 3, 5, 6 and 8 there is a great increase in the amount of fat within the lobule around the central vein, and the drops are very large.

Curative diet animal: Figure 9. The diet was supplemented with tryptophan for seven days after a period of deficiency (ten days). The distribution of fat is similar to that in stock or control animals.

method, large and extremely beautiful preparations were secured representing all four groups of animals:

1. Stock Animals: The mitochondria were mostly exceedingly small and spherical, decreasing in size to the limits of visibility. They were quite numerous and were somewhat scattered in the cytoplasm (fig. 14).

2. Control Animals: The mitochondria were small and spherical and tended to take a peripheral location in the cell. They were less

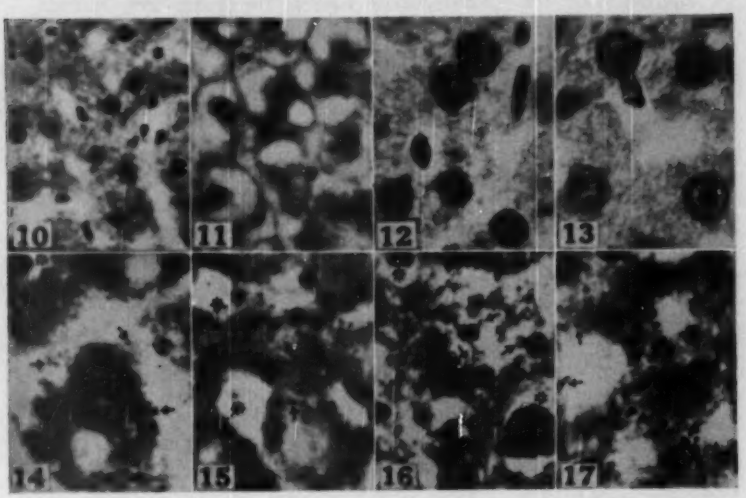


Fig. 10.—Cells of hepatic cords of normal stock animal. Harris' hematoxylin, orange G; × 400.

Fig. 11.—Cells of hepatic cords showing large fat vacuoles. The animal was fed the deficient diet for fifteen days. Harris' hematoxylin, orange G; × 400.

Fig. 12.—Nuclei of normal rat liver cells containing several nucleoli. Harris' hematoxylin, orange G; × 950.

Fig. 13.—Nuclei of tryptophan-deficient rat liver cells containing each a single large nucleolus. Harris' hematoxylin, orange G; × 950.

Figs. 14 to 17.—Liver cells showing the form and the arrangement of the mitochondria. Representative mitochondria are indicated by arrows; fat droplets, by asterisks. In cytologic preparations, mitochondria stain red, whereas fat retains the black color produced by osmic acid; both appear black in the photographs. Altmann's picric acid–fuchsin stain; × 950.

Stock animal: Figure 14. The mitochondria are spherical and very small, decreasing in size to the limits of visibility.

Control animal: Figure 15: The mitochondria are small and spherical and are located near the boundaries of cells. The large round black bodies are fat droplets.

Tryptophan-deficient animal: Figure 16. The mitochondria are larger and vesicular in appearance. Cell structure is not easily recognized, because nearly all fat, particularly that in many of the largest drops, was extracted during dehydration and clearing of the section. The cytoplasm appears stringy, with mitochondria scattered along it.

Curative diet animal: Figure 17. The diet was supplemented with tryptophan for seven days after ten days of deficiency. The mitochondria are similar to those of the control animals but larger and varying in shape from small spheres to short filaments and bent rods. Some large fat droplets remain.

numerous than in the liver cells of stock animals but were more prominent because of their noticeably larger size (fig. 15).

3. Tryptophan-Deficient Animals: The mitochondria were fairly numerous but less so than in the controls. They showed a considerable range in size and shape from very small spherical types to large, irregularly rounded vesicular forms (fig. 16).

4. Curative Diet Animals: The mitochondria were small and varied in shape from spherical to short elongate rods or filaments. These were scattered in the cytoplasm and were about equal to the controls in number (fig. 17).

The relation of the mitochondria and the hepatic fat turnover is not entirely understood as yet, nor is there complete agreement on it. The work of Kater and Smith [4] indicated that fat was formed within the mitochondria, and Kater [4] ventured the opinion that the mitochondria were the centers of enzyme activity associated in some way with fat metabolism. The earlier investigations of Noel [5] also indicated a connection between mitochondria and fat formation, but he believed that fat accumulated outside the mitochondria. Both views have been supported at various times by other investigators, and one of us (see Adamstone and Taylor [5a]) has obtained evidence from studies of frozen sections of hamster liver which appears to support the views of Kater.

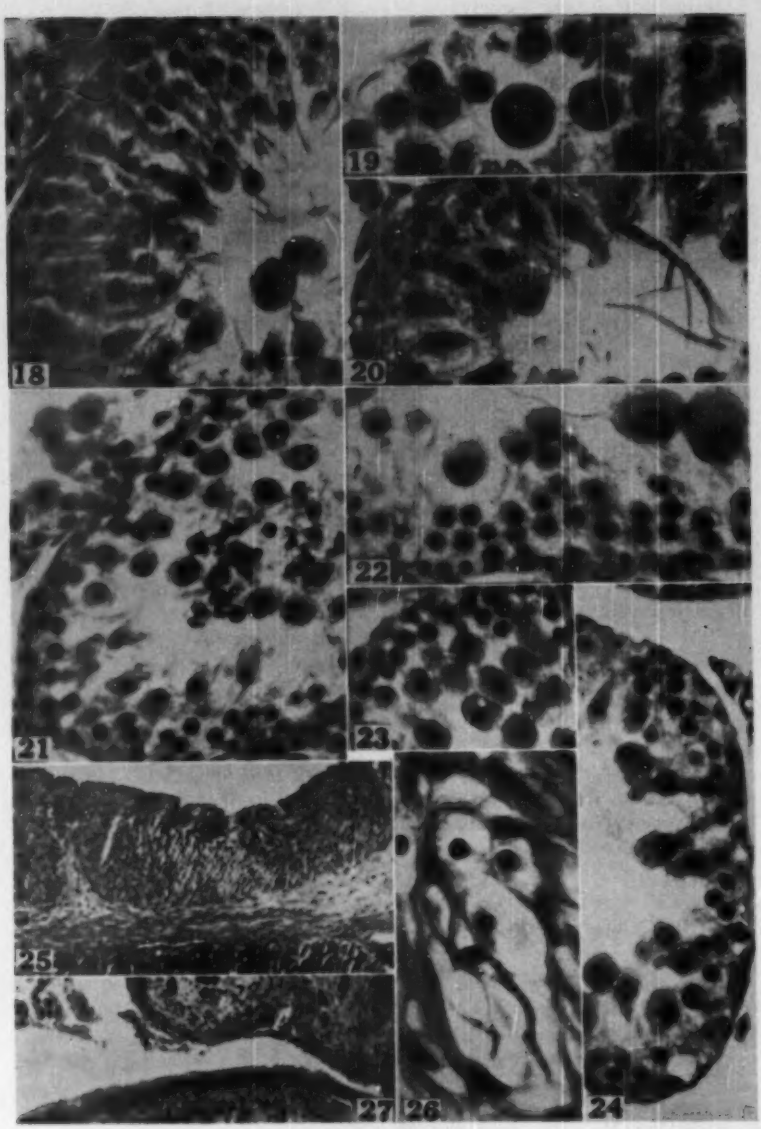
It is obvious from the present studies that tryptophan deficiency in some way interferes with the mechanism of fat turnover in the liver. Just how this occurs is not apparent, but some abnormality of the enzyme system responsible for fat metabolism may be involved. There seems to be considerable likelihood that the mitochondria are associated with the enzymatic activity involved in the synthesis and the degradation of fat as shown by the application of the Gomori technics for the demonstration of lipase and phosphatase activity (unpublished data). The possibility exists, therefore, that the change observed in the mitochondria of the experimental animals may be related in some way to a disturbance of enzyme activity resulting in excess accumulation of fat.

TESTES

In addition to the liver, the testes of male rats used in this experiment showed characteristic degenerative changes even though the period of tryptophan deficiency was of short duration. These changes appeared to follow each other in a fairly definite and orderly sequence, although there was considerable overlapping of stages. Certain aspects of the process appear to be of diagnostic value, although in a general way the

4. Kater, J. McA., and Smith, D. M.: Anat. Rec. **52**:55, 1932.

5. Noel, R.: Arch. d'anat. micr. **19**:1, 1923.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whole process is highly reminiscent of the degeneration of the testes in vitamin E–deficient rats (Mason [6]).

Before any real injury was noticeable in the seminiferous tubules, multinucleate giant cells were occasionally observed in them (fig. 18), followed by pyknosis of primary spermatocytes (fig. 19). The cells of the peripheral zones of the tubules were apparently quite normal, and, depending on the stage of maturation, the usual succession of cell types was recognizable, including spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa, as well as Sertoli cells.

Following the appearance of abnormal primary spermatocytes, fusion of mature spermatozoa occurs in some of the tubules, producing enormous giant sperm cells. In some of these the heads still remain separate and the tips of the tails are also free. Fusion apparently affects the middle piece of the maturing sperm before the rest of the cell (fig. 20).

This is followed by a stage in which individual cells, mostly spermatids and secondary spermatocytes, are sloughed off into the lumen of the tubules in great numbers, where they undergo disintegration (fig. 21). In these degenerating cells the cytoplasm apparently undergoes dissolution first, followed by breaking up of the nuclei.

The stage just described is followed by the formation of giant cells, by fusion of the spermatids, and also by fusion of the secondary spermatocytes. Giant cells of both types appear in the lumen of the tubules in considerable numbers and may be readily recognized by the size of the nuclei contained within them. The number of cells fused to form a single giant cell varies considerably, ranging from 2 to 8 or more (fig. 22).

6. Mason, K. E.: Am. J. Anat. **52**:153, 1933.

Figs. 18 to 27.—All sections were stained with Harris' hematoxylin-eosin.

Fig. 18.—Testis of tryptophan-deficient rat. Several giant cells are present in the lumen of the tubule. Peripheral maturation stages are apparently normal. × 400.

Fig. 19.—Primary spermatocyte enlarged, rounded and deeply stained. It contains a heavily stained nucleus. × 950.

Fig. 20.—Fused sperm cells. Heads and tips of tails are still separate. Middle pieces are fused. × 400.

Fig. 21.—Cellular debris in the lumen of a seminiferous tubule. It is composed of sloughed cells. × 400.

Fig. 22.—Giant cells composed of secondary spermatocytes (large nuclei) and spermatids (smaller nuclei). × 400.

Fig. 23.—Sloughing of primary spermatocytes. × 400.

Fig. 24.—Sloughing of spermatogonia, leaving denuded areas on the basement membrane. × 400.

Fig. 25.—Wall of intestine showing muscle degeneration—lightly stained area with isolated fibers. × 95.

Fig. 26.—Individual muscle cells of the wall of the urinary bladder showing disintegration of fibrils. × 950.

Fig. 27.—Sloughing of eipthelium of urinary bladder. × 95.

The next stage is represented by the sloughing of the primary spermatocytes into the lumen of the tubule (fig. 23). These cells enlarge to a considerable extent, round up and then separate from each other as they are set free in the lumen of the tubule. At this time there is apparently a general loss of cohesion between cells.

This condition of loosening and separation of the cells ultimately affects the spermatogonia, and these also begin to slough off into the lumen of the tubule, leaving areas of the basement membrane denuded of the germinal epithelium. Small areas where this has begun to occur can be seen in figure 24.

Finally the Sertoli cells are left isolated but still attached to the basement membrane. The ultimate effect of this process of degeneration of the seminiferous tubules is the complete removal of the developing germ cells. If this process were allowed to continue to its ultimate conclusion, the males must eventually become sterile. Mating experiments would have to be carried out to prove this point, but the condition of extreme debility characteristic of these animals would probably make mating impossible.

MUSCULAR TISSUE

Both types of involuntary muscle, namely, visceral and cardiac, showed definite reactions to tryptophan deficiency. This was apparent in the muscular layers of the walls of the digestive tube, in the urinary bladder and in the uterus. Areas where degenerative changes had taken place were readily recognized because they had a very light staining reaction and the individual muscle fibers were shrunken and separated, giving the tissue a somewhat shredded appearance (fig. 25). In localized areas of the wall of the urinary bladder complete degeneration of the myofibrils had occurred, so that the nuclei of the muscle fibers were suspended in a mass of lightly staining cellular debris (fig. 26). In cardiac muscle the separation of fibers produced a shredded appearance similar to that seen in the wall of the gut.

In voluntary skeletal muscle, on the other hand, no evidence of degenerative changes was observed.

EPITHELIAL TISSUE

In 3 animals the epithelial lining of the urinary bladder was sloughed off, and remnants of it were found in the lumen (fig. 27). This condition was not extensive, and no evidence of it was found in stock or control animals.

CORNEA OF THE EYE

Thickening and keratinization of the cornea of the eye occurred in more advanced cases of tryptophan deficiency (nine days). Buschke[7]

7. Buschke, W.: Arch. Ophth. **30**:751, 1943.

has made an extensive study of ocular lesions and has described the occurrence of similar ocular lesions in tryptophan-deficient rats accompanied by increased vascularity and the formation of cataracts. Sydenstricker and associates [8] also noted corneal reaction with diets deficient in various amino acids.

COMMENT

Comparison of Tryptophan-Deficient Chick and Rat.—Brown and associates [9] have reported the results of a histologic study of tissues of tryptophan-deficient chicks. These animals were 10 days old at the beginning of the experiment and survived only fourteen to eighteen days. Changes were noted in various tissues, including liver, testes, spleen and muscle. In the liver the cells showed lightly stained cytoplasm containing large vacuoles. This condition was interpreted as "exhaustion of the cell contents." It appears more probable, however, in view of the present findings in the rat, that the conditions described are the result of a disturbance of fat metabolism, with fat accumulating in the liver cells in consequence. In the testes of the chick, degeneration of the germinal epithelium was observed. This also occurred in the rat. As for the spleen, no changes were observed in the rat, but certain degenerative changes were found in the chick. The voluntary muscle of the rat showed no demonstrable effects of tryptophan deficiency, but atrophy occurred in both smooth and cardiac muscle. In this the rat contrasts with the chick, in which skeletal muscle shows atrophy. Brown [9] also described erosion of the mucosa of the gallbladder of the chick, a structure which is lacking in the rat. There was, however, evidence of epithelial sloughing in the lining of the urinary bladder of the rat. This suggests that epithelial tissues are susceptible to injury by tryptophan deficiency.

The results of this study indicate that a series of characteristic pathologic changes occur in the tissues of tryptophan-deficient rats. These changes have not been exhaustively studied in rats by other workers except for the occurrence of cataract and keratinization of the cornea, which was described by Buschke [7] in 1943. It has also been stated that atrophy of the testis and aspermiogenesis may occur in these rats (Albanese and Buschke [10]). Furthermore, Keller [11] claimed that sterility was induced by tryptophan deficiency in rats, but no histologic

8. Sydenstricker, V. P.; Hall, W. K.; Bowles, L. L., and Schmidt, H. L., Jr.: J. Nutrition **34**:481, 1947.

9. Brown, S. O.; Wilkening, M. C., and Schweigert, B. S.: Proc. Soc. Exper. Biol. & Med. **68**:672, 1948.

10. Albanese, A. A., and Buschke, W.: Science **95**:584, 1942. Albanese, A. A.: Science **101**:619, 1945.

11. Keller, W. F.: Science **103**:137, 1946.

evidence was adduced. On the contrary, Berg and Rohse [12] were convinced that after a period of twenty days tryptophan deficiency did not produce subsequent sterility. The results of the present histologic study indicate that destruction of the germinal epithelium and sterility would probably occur if the animals survived long enough. It would also be expected that sterility would be produced in the chick on the basis of Brown's [9] results.

The findings of a histologic study of the liver show that a disturbance of fat metabolism is induced which results in a great accumulation of fat in the liver. This effect is apparently not produced by any other amino acid deficiency.

The degeneration of muscular tissue appears to be a direct result of lack of tryptophan for maintenance of muscle proteins, but the explanation of the epithelial desquamation is not apparent.

SUMMARY AND CONCLUSIONS

Tryptophan deficiency induced in the rat by forced feeding of a purified tryptophan-deficient ration produces a series of pathologic alterations, among which the following are prominent and characteristic:

1. Heavy accumulation of fat in the liver, resulting in vacuolation of the cytoplasm of the liver cells. With this change, a single large nucleolus appears in the nucleus, in contrast with the normal nucleus, in which several nucleoli are usually present.

2. Degeneration of the seminiferous tubules. This is accompanied by the formation of giant cells and the sloughing of cellular elements as a result of loss of cohesion among the primary spermatocytes. The rounding-up of the primary spermatocytes and the great increase of the affinity of these cells for stains mark the beginning of the degenerative changes in the testis.

3. Atrophy and degeneration of visceral and cardiac muscle. This is a striking effect of tryptophan deficiency.

4. Keratinization of the cornea. This process is recognizable after about nine days of tryptophan deficiency and is usually accompanied by other ocular lesions, which have been adequately described by Buschke.[7]

5. Injury of epithelial tissues. This was observed in a few organs, but its importance appears to be limited.

12. Berg, C. P., and Rohse, W. G.: Science **105**:96, 1947.

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POTASSIUM DEFICIENCY SYNDROME IN THE RAT AND THE DOG

A Description of the Muscle Changes in the Potassium-Depleted Dog

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AND

THOMAS E. LASATER

DURHAM, N. C.

MANY investigators[1] have observed the effects of potassium-deficient diets on rats. A mass of somewhat conflicting evidence has been presented, but the general consensus is that diets containing 0.01 per cent or less of potassium are growth limiting. Death occurs usually in from twenty-one to thirty-five days, the deficiency resulting in fairly characteristic pathologic changes in heart and kidney. No paralysis or demonstrable skeletal muscle changes have been reported.

On the other hand, in the dog either the direct dietary[2] or the indirect hormonal[3] production of potassium deficiency results regularly in the development of a series of paralytic attacks from which the animal recovers spontaneously, followed by progressive paralysis which, if untreated, eventually results in death. In our earlier studies a specific reversal of the progressive process resulted when 1 mg. of biotin was given subcutaneously. Potassium chloride failed at a dose level of 700 mg. but was effective in doses of 1 to 5 Gm. The minimum effective

From the Departments of Neuropsychiatry and Pathology, Duke University School of Medicine.

1. (*a*) Osborne, T. B., and Mendel, L. B.: J. Biol. Chem. **34**:131, 1918. (*b*) Miller, H. G.: ibid. **55**:61, 1923. (*c*) Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: J. Nutrition **14**:85, 1937. (*d*) Grijns, G.: Ztschr. f. Physiol. Chem. **251**:97, 1938. (*e*) Heppel, L. A., and Schmidt, C. L. A.: Univ. California Publ., Physiol. **8**:189, 1938. (*f*) Thomas, R. M.; Mylon, E., and Winternitz, M. C.: Yale J. Biol. & Med. **12**:345, 1940. (*g*) Orent-Keiles, E., and McCollum, E. V.: J. Biol. Chem. **140**:337, 1941. (*h*) Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: Am. J. Path. **18**:29, 1942. (*i*) Follis, R. H., Jr.: Am. J. Physiol. **138**:246, 1943. (*j*) Skinner, J. T., and McHargue, J. S.: ibid. **143**:385, 1945. (*k*) Kornberg, A., and Endicott, K. M.: ibid. **145**:291, 1946.

2. (*a*) Smith, S. G.: Am. J. Physiol. **144**:175, 1945. (*b*) Ruegamer, W. R.; Elvehjem, C. A., and Hart, E. B.: Proc. Soc. Exper. Biol. & Med. **61**:234, 1946. (*c*) Smith, S. G.: ibid. **63**:339, 1946.

3. Ferrebee, J. W.; Parker, D.; Carnes, W. H.; Gerity, M. K.; Atchley, D. W., and Loeb, R. F.: Am. J. Physiol. **135**:230, 1941.

therapeutic dose of potassium chloride used in this laboratory was 200 mg. per kilogram of body weight. At this dose level, potassium proved much more effective than biotin, and it was concluded that potassium rather than biotin was the primary dietary deficiency responsible for the paralytic syndrome observed.

To our knowledge the pathologic anatomy of the lesion in the dog has not yet been reported. It is the object of our communication to report this and to compare the symptoms and the pathologic changes of the two species fed the same diet.

EXPERIMENTAL PROCEDURE

Rats.—*General Scheme.*—In constructing a deficient diet the ideal is to arrive at one lacking a single nutrient substance and known to be adequate in all others. This is impossible to achieve with respect to the vitamin B complex. There are two alternatives; one is to use only the synthetic factors as a source of the B complex, and the other is to supply this complex by the use of natural concentrates, such as brewers' yeast, rice polish, wheat bran, etc. Neither of these alternatives is entirely satisfactory. In the first instance, we know exactly the vitamin B content of the diet and are reasonably sure that the factors included are supplied in adequate amounts, but there are inevitable deficits of unknown and unsynthesized factors. In the second case, although we have more of the B complex factors present, the exact content is not known, and the possibility still remains that some are lacking altogether or are present in such low concentrations that they are ineffective in the diet. The uncertain composition of this diet militates against it; therefore, we have chosen the first alternative. The exact components of diets A, B, C, D and E used in these experiments for both species are given in tables 1 and 2. The first four (A, B, C and D) differ only in mineral content, while diet E is an attempt to arrive at a completely adequate diet through the use of brewers' yeast as a source of both the B complex vitamins and potassium.

Forty-nine weanling albino rats of the Osborne-Mendel strain were divided into four groups. Group 1 received diet A, which has a potassium content of 0.01 per cent or less. Group 2 received diet B, identical with A except that potassium was introduced into the salt mixture, giving a level of between 0.23 and 0.26 per cent (average 0.24) per cent) in the diet. This level should be entirely adequate for a potassium control diet, since Kornberg and Endicott [18] have shown that a diet containing 0.17 per cent potassium not only prevents characteristic lesions but results in optimal growth. The level used in these experiments regularly prevents paralysis in the dog. Group 3 received diet C and later D, identical with B except that the potassium content was, respectively, two and three times that of diet B. Group 4 received diet E in which both the potassium and the B complex factors were supplied by brewers' yeast, fed at a level of 10 per cent. This diet, containing between 0.22 and 0.26 per cent (average 0.24 per cent) potassium, proved entirely adequate with respect to growth, reproduction and general physiologic well-being of the animals, though evidence obtained in this experiment indicates that an unknown lactation factor (or factors) is either inadequate or present in very minimal amounts.

Pathologic Technic.—Tissues from 8 deficient rats (diet A), 2 potassium controls (diet B) and 1 yeast control rat [4] (diet E) were prepared and examined, only

4. One animal seems inadequate, but at least 25 such controls have been examined in other experiments, and in every case the tissues were normal.

TABLE 1.—*Diets*

Ingredients	A*	B	C	D	E
Casein (acid and alcohol extracted)	40	40	40	40	40
Sucrose	44	44	44	44	34
Cotton seed oil	10	10	10	10	10
Cod liver oil	2	2	2	2	2
Salt mixture A	4	..	..	..	4
B	..	4	..	..	..
C	..	..	4	..	..
D	..	..	..	4	..
Brewers' yeast	..	..	..	..	10
	100	100	100	100	100

Vitamin supplements:	Rat per Day	Dog per Day
1. Fat-soluble vitamins:		
Vitamin K, menadione	0	0.7 mg.
Vitamin E, mixed tocopherols	0.2 mg.	35 mg.
Vitamin A } cod liver oil concentrate (Lederle)	250 U.S.P. units	20,000 U.S.P. units
Vitamin D }	25 U.S.P. units	2,000 U.S.P. units
2. Vitamin B complex factors:	Micrograms per Day	Mg. per Day
Thiamine	20	1.4
Riboflavin	50	0.7
Pyridoxine	100	6.0
Nicotinic acid	100	6.0
Pantothenic acid	100	6.0
Para-aminobenzoic acid	100	6.0
Inositol	5 mg.	100.0
Choline	6 mg.	100.0

All vitamin supplements were dissolved either in water or in oil, according to their solubilities, and were fed individually to each animal weekly.

* Late in the experiment, the sucrose was increased from 44 to 64 per cent at the expense of the casein, with the idea of shortening the period required for paralysis to develop. It proved to be of little value from this standpoint. The potassium content was very slightly lower, 0.008 to 0.009 per cent.

TABLE 2.—*Salt Mixtures*

Ingredients	A	B	C	D
Bone meal (steamed) *	57.5	57.5	45.5	21.2
Sodium chloride	26.4	13.2	13.2	13.2
Potassium chloride	....	13.2	24.4	49.8
Limestone †	13.2	13.2	13.2	13.2
Iron sulfate	3.7	3.7	3.7	3.7
Magnesium oxide	1.2	1.2	1.2	1.2
Copper sulfate	0.3	0.3	0.3	0.3
Manganese sulfate	0.1	0.1	0.1	0.1
Zinc oxide	0.1	0.1	0.1	0.1
Cobalt carbonate	0.1	0.1	0.1	0.1
Potassium iodide	0.1	0.1	0.1	0.1
	100.0	100.0	100.0	100.0

* This was substituted late in the experiment by monohydric orthophosphate ($CaHPO_4$).
† This was substituted late in the experiment by calcium carbonate ($CaCO_3$).

the hematoxylin and eosin stain being used. Cross sections of the tails of 5 yeast controls, 8 potassium controls, and 1 potassium-deficient animal were prepared in the same manner.

Potassium Assay.—(*a*) Diet: Accurately weighed samples (approximately 2 Gm.) were placed in a platinum crucible, and 1 cc. of four times-normal sulfuric acid was added per gram of diet and the mixture charred over a water bath, according to the method of Hald.[5] The crucible was then placed in a cold muffle oven and the temperature brought gradually to 500-550 C. and maintained at this temperature over night or until a white ash remained. To the ash 1 cc. of concentrated hydrochloric acid was added for each gram of food represented and the dish warmed to facilitate solution of the ash. With the aid of distilled water, the contents of the crucible were transferred quantitatively to a volumetric flask. Sufficient lithium was added to give a resulting concentration of lithium of 400 p. p. m. The mixture was then diluted to a suitable volume and read according to the internal standard method on the flame photometer (model 52-A, Perkin-Elmer Corporation). Ten or more readings were taken on each sample.

(*b*) Serum: Blood was collected under oil with absolutely no hemolysis. Whenever there was any question, the specimen was discarded. Sufficient lithium was added to bring the concentration to 400 p. p. m. The sample was then diluted 1 to 50 or 1 to 25, according to the suspected potassium concentration, and read in the flame photometer. If the 1 to 50 dilution is used, the sodium can be read on the same solution.

Dogs.—*General Scheme.*—Twenty-five dogs have been studied since 1945.[2a] Twenty were restricted to the deficient diet A (table 5), 4 to the potassium-supplemented diet B and 1 to the yeast-supplemented diet E (table 6), which is completely adequate for growth, general physiologic well-being and reproduction in the rat.

Pathologic Technic.—Owing to circumstances beyond our control, tissues were not available for study from all animals; thus, material from 13 deficient animals (diet A), 1 potassium control (diet B) and 5 yeast controls (diet E) was studied. Skeletal muscle was the only tissue examined from 4 of the deficient and 5 of the control dogs. From the other animals the following tissues were examined: heart, lung, spleen, liver, kidney, adrenal gland, pancreas, stomach and skeletal muscle. The tissues for microscopic study were prepared with hematoxylin and eosin stain. In some cases, brain, spinal cord and peripheral nerve tissue were sectioned.

The neuropathologic material was obtained from 3 potassium-deficient and 2 yeast control dogs. The spinal cords of all and the brains of 4 animals were examined at various levels by means of the following stains: hematoxylin and eosin, cresyl violet and the Marchi, Kulschitsky and Weil methods for studying myelin degeneration.

Dr. Talmadge Peele arranged for these tissues to be prepared in his laboratory and supervised their preparation.

RESULTS

Rats.—*General or Clinical Observations.*—The weanling rats, 4 weeks old (table 3, groups 1A and 1B), soon ceased to grow on diet A and died within an average of three weeks when caged individually. It is not accurate to judge the life span by means of rats caged in groups where casualties occur, because rats tend to consume their deceased mates and this, of course, changes the nutritional

5. Hald, P. M.: J. Biol. Chem. **167**:499, 1947.

status of the survivors. However, when the potassium-depleted weanling rats were given diet B at the end of three weeks, they rapidly gained weight and soon matched that of their littermates receiving this diet from the beginning of the experiment.

Older rats, 19 weeks old (group 1C), receiving diet A continued to grow for a much longer period and survived an average of twenty-eight weeks. When the experiment was terminated in eight weeks, there was little difference in the appearance of those rats whose diet was supplemented with potassium (diets B, C and D) and those with yeast (diet E). However, when the experiment was continued for forty-three weeks or more, a marked difference occurred, particularly in the coat. This increased experimental period permitted the appearance of certain secondary deficiencies in the diet—in this instance, probably factors of the vitamin B complex. On the basic diet A and the potassium-supplemented diets B and C (fig. 1 *A*, *B* and *C*), the coat becomes very greasy and discolored, and there are various degrees of alopecia; whereas in the rats on the yeast-supplemented diet E, which supplies adequate amounts of both potassium and B complex factors, the

TABLE 3.—*Rats Fed Deficient and Control Diets*

Group	Rats	Initial Age, Weeks	Diet (Ash, %)	Approx. Content of Potassium, %	Initial Weight, Gm.	Final Weight, Gm.	Av. Longevity on Diet, Weeks	Duration of Experiment, Weeks	Caging	Comment
1 A	15	4	A (4.91)	0.009*	51	89	(5)†	8	Group	Coat very sticky and dirty
B	5	4	A	.....	45	48	8	8	Individual	Coat very sticky and dirty
C	41	19	A	.....	284	231	(28)†	28	Group	Coat very sticky and dirty
2 A	5	4	B (4.56)	0.24*	54	191	8	8	Group	Coat clean
B	5	4	B	.....	46	249	43	43	Group	Coat sticky
3	5	4	C—D	.....	44	414	(39)†	43	Group	Coat sticky
4 A	5	4	E	0.24*	54	278	8	8	Group	Coat clean and healthy
B	5	4	E	.....	44	467	43	43	Group	Coat clean and healthy

* This is the average value from the assay of 4 samples.

† The value in parentheses is the average for rats caged in a group in which death occurred during the experiment.

‡From the time of weaning (4 weeks) these rats were restricted to diet B for fifteen weeks; then they were given diet A.

coat remains clean and normal in appearance (fig. 1 *D*). Paralysis was not observed in any of the rats.

There was a sharp difference in the reproductive capacity of the rats fed the different diets. There was no reproduction in the females of groups 1 and 2. The 2 females of group 3 had one litter each of 4 pups, all dying within twenty-four to seventy-two hours; the 2 females of group 4 had five and six litters, respectively, each litter ranging from 5 to 11 pups, all of whom were healthy and normal at birth. The pups of all litters of 1 rat died within twenty-four to seventy-two hours, owing to inadequate lactation, while those of the other female in group 4, having six litters, lived until put to death at 3 weeks of age. This animal was able to suckle her young and maintain them in health.

Pathologic Anatomy.—The pathologic changes brought about by the potassium deficiency in the rats are identical with those reported by other investigators,[6]

6. Schrader and others.[1c] Thomas and others.[1f] Follis and others.[1h] Follis,[11] Kornberg and Endicott.[1k]

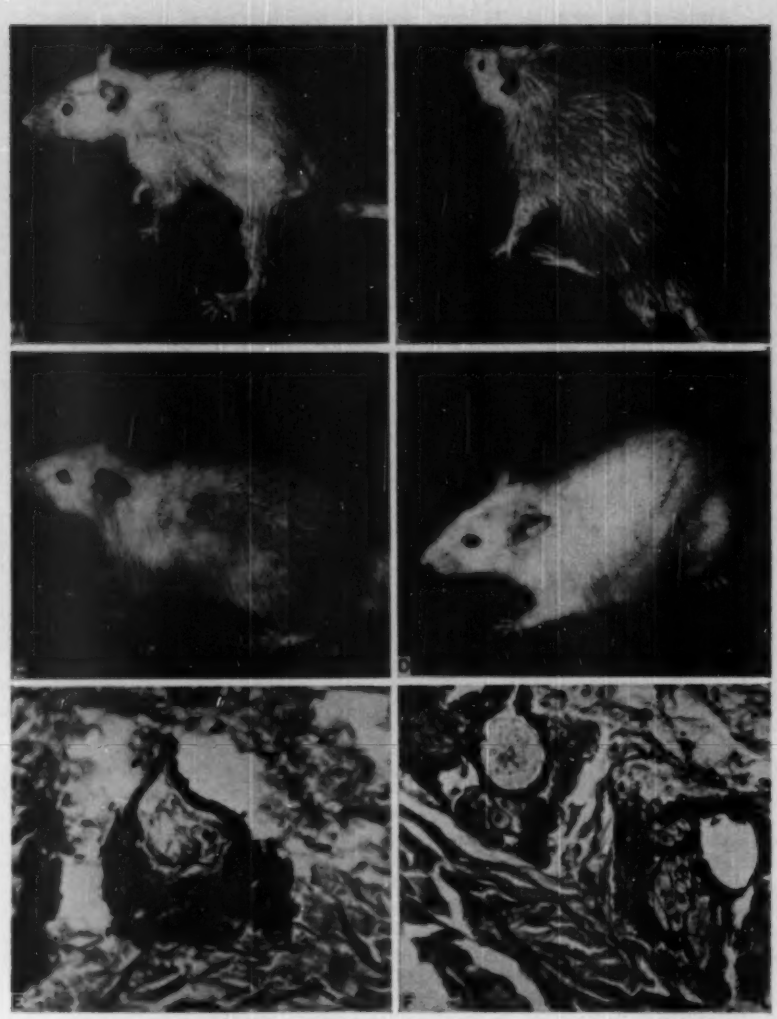

Fig. 1.—*A*, rat on diet A (containing 0.01 per cent potassium). *B*, rat on diet B (containing 0.24 per cent potassium). *C*, rat on diet D (containing 1.042 per cent potassium). *D*, rat on diet E (containing 0.24 per cent potassium supplied by brewers' yeast). *E*, cross section of the tail of a rat on the same regimen as the rat shown in *B*. *F*, cross section of the tail of a rat on the same regimen as the rat shown in *D*.

namely, myocardial necrosis followed by fibrosis (fig. 2 *A*) and dilatation of the renal loops of Henle (fig. 2 *B*). No lesions attributable to the deficiency could be recognized in skeletal muscle, smooth muscle or viscera other than the heart.

The cross sections of the tails of the potassium controls having sticky coats (table 3, groups 2 B and 3; fig. 1 *B* and *C*) showed changes in the sebaceous glands. Atrophy was present in all. The individual cells were reduced in size, leading to a decrease in the size of the glands (fig. 1 *E*). The cells possessed a more homogeneous pink-staining cytoplasm in contrast to the vacuolated normal appearance. In the most extreme cases sebaceous glands could be recognized only in the form of a few small pyknotic cells associated with hair follicles. The sebaceous glands of all 5 yeast controls with clean, healthy coats (table 3, group 4 B; fig. 1 *D*) were normal in number and appearance (fig. 1 *F*).

Dogs.—*General or Clinical Observations.*—The dogs, like the rats fed diet A, showed distinct slowing of their growth rate, followed by actual loss of weight. In contrast to the rats, the dogs became paralyzed. Fifteen of the 20 dogs dying

TABLE 4.—*Reproductive Performance of Rats*

Group	Females	Age at 1st Pregnancy, Weeks,	Litters	Pups per Litter	Longevity of Pups	Potassium Content of Diet, per Cent	Comment
1 C	2	..	0	0	.....	0.000	No reproduction; no lactation
2 B	2	..	0	0	.....	0.205	No reproduction*; no lactation
3 B	2	29	1	4	Only a few hours	1.042	Reproduction below par
		32	1	4	Only a few hours		No lactation
4 B	2	18	6	5, 11, 5, 7 (?) 9	Lived until killed	0.129	Reproduction and lactation normal
		19	5	5, 7, (?) (?) 10	Died in 24 to 72 hours		Reproduction normal; lactation nil

* Further experiments with more females indicated that reproduction occurs at this level of potassium when the animals are placed on the diet after sexual maturity but that lactation is entirely inadequate.

on diet A had 21 attacks of the characteristic progressive paralysis, thus confirming our earlier work. Of the 5 dogs dying without symptoms of progressive paralysis, 2 had changed areas in the lung, thought to be early pneumonia, and the other 3 died suddenly of unknown cause. The paralysis seen in these dogs resembled periodic paralysis in man. It was associated with greatly reduced serum potassium values, while the serum magnesium, calcium and sodium values remained essentially unchanged (table 5).

An analysis of the cause of death in the 15 dogs suffering paralytic attacks is of interest. In 2 untreated animals the paralysis progressed to respiratory failure and death. In the untreated dogs with progressive paralysis death was always due to respiratory failure. Two dogs died as a result of potassium chloride therapy—one from aspirating an oral dose and the other from too rapid intravenous injection. Two others may have died of paralysis, but all the 9 remaining ones died very suddenly of unknown cause in the interval between attacks of paralysis. Most of them had pulmonary edema and often acute passive congestion of the liver, strongly suggestive of cardiac failure. Electrocardiograms taken on most of these dogs varied somewhat but showed a picture less severe perhaps but similar to that

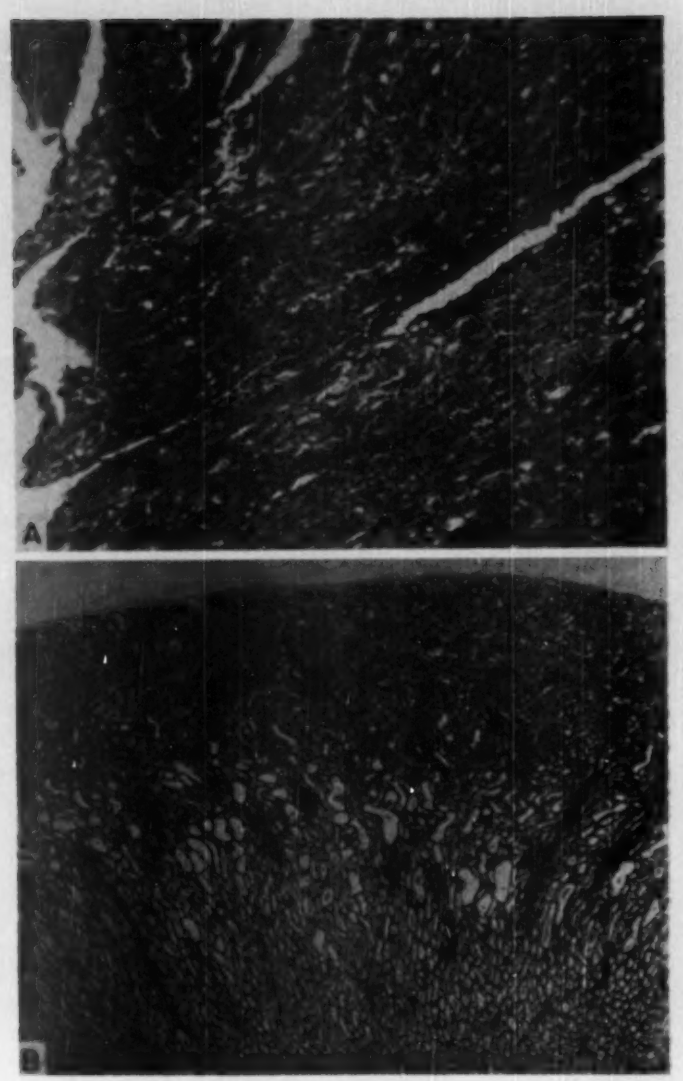

Fig. 2.—Potassium-deficient rat tissue: *A*, fibrosis and round cell infiltration following necrosis of the myocardium of the left ventricle. *B*, cystic dilatation of the renal loops of Henle.

observed in cases of familial periodic paralysis[7] and other potassium deficiency syndromes.[8] These data are at present being prepared for publication.

The deficient dogs (diet A) have also shown a rather definite increase in their fluid exchange as compared with that of the controls (diets B and E). This was not true of the rats to any significant extent. It was not unexpected, since others[9] had observed that "a diabetes insipidus-like condition" develops in dogs given desoxycorticosterone. These observations are being reported in more detail elsewhere.

Yeast is apparently a more adequate supplement to the basic diet than potassium. Four of the 5 dogs on diet B showed poor and irregular growth and diet from

TABLE 5.—*Dogs Receiving Diet A with a Potassium Content of 0.01 Per Cent*

Dog	Initial Age, Weeks	Initial Weight, Kg.	Weight Gain, Kg.	Serum Potassium, Mg. per 100 Cc.*	Time on Experiment to Paralytic Attack, Weeks	Attacks of Paralysis	Therapeutic Supplement	Dose, Mg. per Kg.	Fate (See key below)	Survival, Weeks
167	17	6.4	—0.5	.....	18	1	None	...	D.P.	18
169†	45	13.6	—2.2	11.3 (P) 15.0 (R)	89, 10 2, 5	4	(1) Biotin, KCl (2) Biotin (3) KCl (4) KCl	0.3, 500 0.24 500 200	D.S.C.U. (P.E.)	87
170	21	5.9	+0.2	.....	14	1	None	...	D.P.	..
172†	47	12.9	—2.9	.....	..	2	None	...	D.P.	25
173	20	7.7	0.0	.....	7	1	KCl	625	D.S.C.U. (P.E.)	9
175	20	5.5	—1.2	12.7 (P)	21	1	(1) Biotin, KCl (2) KCl	1.2, 264 200	D.S.C.U. (P.C.L.)	37
176	21	9.1	—1.8	11.5 (P)	12	1	Biotin sub.	0.24	D.S.C.U. (P.E.) (P.C.L.)	14
178	18	4.3	+0.5	.....	4	2	(1) KCl, PO (2) Biotin, KCl	600 0.32, 200	D.S.C.U.	19
179	15	2.7	—0.5	12.4 (P)	13 5½	2	(1) KCl, PO KCl (I.V.)	200 45	D.R.K.	18
180	14	3.2	—0.5	.....	..	0			D.Pn.	17
181	14	3.0	—1.3	.....	..	0			D.Pn.	20
183	14	2.7	—1.9	.....	12	1	Biotin KCl	1.7 200	D.S.C.U.	18
184	14	2.7	—0.2	10.5 (P)	16	1	KCl (I.V.)	80	D.A.K.	20
185	15	4.6	—0.5	.....	14½	1	KCl	200	D.S.C.U.	23½
187	14	2.7	—0.2	.....	..	0			D.S.C.U.	20
188	14	2.7	—0.2	.....	..	0			D.S.C.U.	22
189	21	5.5	—1.2	12 (P)	13½	1	KCl (I.V.)	80	D.S.C.U.	13½
190	22	6.8	.....	8.9 (P)	..	1	KCl PO	200	D.P. (?)	21½
191§	16	5.7	—2.5	9.8(R)	..	?				
192	18	5.2	—1.3	9.5 (P)	13½	1	KCl PO	200	D.C.U.	34½

* In this column (P) means that the animal had paralysis; (R) that recovery occurred and the serum specimen was taken twenty-four hours after the start of oral administration of potassium.

† The values obtained for these animals were omitted from the averages because of the initial disparity of age.

§ This dog had five severe transient attacks but was never observed in a progressive attack.

Key to fate: D.P. = died of paralysis; D.S.C.U. = died suddenly, cause unknown; P.E. = pulmonary edema confirmed by section; D.R.K. = died from reaction to KCl given intravenously; D.A.K. = died from aspiration of oral dose of KCl given prophylactically four weeks after recovery from attack; P.C.L. = passive congestion of the liver; D.Pn. = died of pulmonary changes thought to be early pneumonia.

unknown cause in from six to twenty-two weeks. This was not averted by increasing the potassium intake. The fifth dog lived longer (one hundred and

7. Talbott, J. H.: Medicine **20**:85, 1941.

8. (*a*) Stephens, F. I.: Ann. Int. Med. **30**:1272, 1949. (*b*) Brown, M. R.; Currens, J. H., and Marchand, J. F.: J. A. M. A. **124**:545, 1944.

9. Mulinos, M. G.; Spingarn, C. L., and Lojkin, M. E.: Am. J. Physiol. **135**:101, 1941. Ferrebee and others.[8]

fifty-seven weeks) but finally died in much the same manner of unknown cause without having undergone paralysis. The hemoglobin content of this dog ranged from 13.3 to 17.5 Gm. in 100 cc. of blood as compared with average contents of 18 to 21 Gm. in 100 cc. of blood shown in dogs of the same age on diet E.[10]

We have had no deaths out of 11 dogs fed the yeast control diet. One has been under constant observation for six years, another for five. Both are still in excellent condition.

Pathologic Anatomy.—In the thoracic and abdominal viscera of the 10 dogs thus examined no lesions ascribable to potassium deficiency could be found. In skeletal muscle, on the other hand, degeneration and necrosis of individual fibers were seen in 11 of the 13 deficient animals (diet A). Of the 2 animals negative in this respect, one (181) failed to present clinical evidence of myopathy before

TABLE 6.—*Controls*

Dog	Initial Age, Weeks	Initial Weight, Kg.	Final Weight, Kg.	Serum Potassium Mg. per 100 Cc.	Survival, Weeks	Attacks of Paralysis	Max. Hemoglobin Content, Gm. per 100 Cc.	Final Age and Fate, Weeks
A. Potassium Control Dogs on Diet B, Containing 0.365 per Cent Potassium								
174	22	6.6	7.7	....	5	0	11.7*	27; died
177	14	5.2	6.6	20.1	157	0	17.5	171; died
182	14	2.7	5.2	....	92	0	13.3	30; died
186	15	3.0	4.2	....	20	0	11.7	35; died
197	20	7.6	4.3	19.4	35	0	13.3	55; died
Average	17	5.0	5.6	19.7	48	0	13.5	66; died
B. Yeast Control Dogs on Diet E, Containing 0.239 per Cent Potassium†								
118	Adult (?)	14	18.0	....	89	0	20.2	1; killed
125	26	7.7	8.4	....	56	0	22.0	44; killed
129	6	3.5	10.0	....	45	0	18.0	53; killed
130	6	3.9	8.8	....	62	0	20.0	70; killed
139	10	2.3	7.0	....	63	0	21.0	73; killed
151	17	5.5	11.0	....	47	0	21.5	64; killed
152	17	5.6	18.0	....	47	0	19.5	64; killed
154	68	5.2	12	17.3	295	0	18.5	353; living
159	21	5.9	8.5	....	61	0	16.8	82; killed
171	17	5.0	10.0	18.1	245	0	19.8	262; living
Average‡	14	5.1	10.5	17.7	..	0	19.5	

* This was the initial value and no other was obtained.

† The average serum potassium level for 18 assays on 6 healthy stock dogs varied from 17.1 to 23.2 mg. per hundred cubic centimeters, with an average of 19.1 mg.

‡ Values of dogs 118, 125 and 154 were omitted from the average because of different age and treatment.

death and the other had convulsive seizures due presumably to a lack of pyridoxine (vitamin B_6) and died very soon after the onset of paralysis.

The involved muscle fibers were characterized by waxy degeneration of their sarcoplasm with loss of striations (fig. 3 *A*, *B* and *C*). The ultimate disruption of the sarcoplasm was accompanied by an increase of cells, invading macrophages and proliferating (regenerating) muscle nuclei (fig. 3 *B*, *C* and *D*). The sarcolemma was intact in most instances, and the surrounding connective tissue and blood vessels appeared uninvolved in the process.

The central nervous systems of 5 dogs revealed no lesions which could be ascribed to potassium deficiency.

10. Smith, S. G.: Am. J. Physiol. **142**:476, 1944.

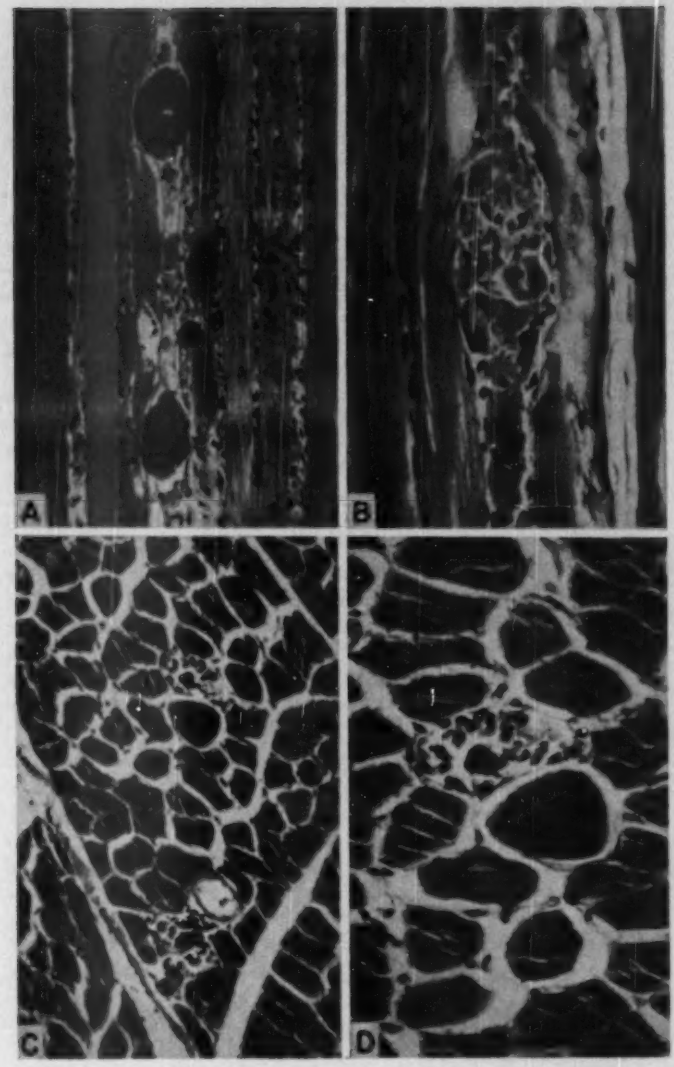

Fig. 3.—Potassium-deficient dog tissue: *A*, Zenker's waxy degeneration of striated muscle showing accumulation of well circumscribed striationless sarcoplasm with intact sarcolemma. *B*, disruption of waxy mass and accumulation of macrophages and regenerating muscle cells. *C*, cross section of similar changes seen in *A* and *B*. *D*, higher power view of field in *C* showing early and later changes of Zenker's waxy degeneration.

COMMENT

The sticky condition of the coats of the rats in groups 1, 2 and 3 could be correlated with characteristic changes in the sebaceous glands but not with a lack of potassium in the diet. These changes have been described before [11] and attributed to a factor (or factors) in the vitamin G complex (heat-stable factors of the vitamin B complex). In a study designed to determine the etiologic factors of the condition, Smith [12] fed riboflavin separately to rats on a diet lacking the whole B complex except thiamine (vitamin B_1) and did not succeed in preventing the sebaceous gland changes. Sullivan and Nicholls,[13] on the other hand, produced the changes with a riboflavin-deficient diet and observed that administration of riboflavin resulted in "a striking regrowth of hair follicles and sebaceous glands." There are several possible explanations of these conflicting results: 1. More than one vitamin factor is necessary for maintaining the integrity of the sebaceous glands. 2. Possibly one of the newer unsynthesized factors of the B complex is specifically involved in maintaining the sebaceous glands but a lack of riboflavin interferes with its utilization or absorption. 3. There is not one or even two specific factors but all are required in a rather nonspecific manner. We are inclined to favor the second hypothesis. In the present study adequate amounts of riboflavin and seven other factors of the B complex were given to each rat individually in adequate amounts, while several other unsynthesized factors of the B complex were missing. Under these conditions the characteristic changes occurred in the sebaceous glands. Although this work was done with a different strain of rat and at a different season of the year, the results entirely confirm those of the earlier study from this laboratory.

A disturbance of potassium metabolism is often associated with paralysis. For example, in periodic paralysis of man [14] the loss of muscle function is associated with a drop in serum potassium and a marked decrease in the potassium excreted in the urine. There is, however, no constant level of serum potassium associated with the onset of the paralysis, but attacks develop on falling values and recovery coincides with rising values. The paralyzed dogs also showed a lowered serum potassium [9c] similar to that seen in cases of periodic paralysis of man.[7] The two syndromes are in fact so similar we have presented evidences

11. Smith, S. G.: Proc. Soc. Exper. Biol. & Med. **30**:198, 1932. Smith, S. G., and Sprunt, D. H.: J. Nutrition **10**:481, 1935. Sullivan, M., and Nicholls, J.: J. Invest. Dermat. **3**:377, 1940.

12. Smith, S. G.: J. Nutrition **15**:45, 1938.

13. Sullivan, M., and Nicholls, J.: J. Invest. Dermat. **4**:181, 1941.

14. Ferrebee, J. W.; Gerity, M. K.; Atchley, D. W., and Loeb, R. F.: Arch. Neurol. & Psychiat. **44**:830, 1940.

that the canine syndrome is the animal analogue of the human.[15] It is of interest here that the paralysis induced in dogs by the intravenous injection of magnesium salts is associated with a drop in serum potassium to approximately the same level found in the potassium-depleted dog.[16]

Paralysis accompanied by decrease of serum potassium has been reported as occurring during the treatment of diabetic acidosis.[17] This has been ascribed to the therapeutic use of large amounts of dextrose, insulin and fluid, resulting in rapid deposition of liver glycogen and establishment of diuresis, and coincidently in a pathologic lowering of serum potassium. Paralysis has also been observed in certain cases of chronic nephritis associated with depletion of serum potassium.[a,b]

In view of the fact that we have obtained therapeutic results with biotin which, though less satisfactory, were yet quite definite, it is interesting that paralysis has been produced in dairy cattle on a biotin-deficient diet which responds to synthetic biotin.[18] Later Flipse and associates[19] produced paralysis in calves which responded both to potassium and biotin and was apparently very similar to that described here.

How potassium deficiency produces muscle dysfunction and degeneration is as yet unexplained. While it has been known that the element plays an important role in the metabolism of muscle, it now appears to be essential to its anatomic integrity.

The canine lesion reflected in the waxy degeneration (Zenker) of skeletal muscle (fig. 3) is similar to that produced by vitamin E deficiency in many species, including rats, rabbits, guinea pigs, dogs and ducklings.[20] Muscle paralysis has also been demonstrated in dogs suffering from a lack of vitamin E. This condition, if not allowed to progress too far, responds to alpha tocopherol.[21] In the experiments reported here vitamin E in the form of the mixed tocopherols was fed individually and in adequate amounts.

Zenker's waxy degeneration has been studied in living muscle by Speidel,[22] who concluded that the phenomenon which he has named "clot formation" may appear within minutes after direct injury and can be

15. Smith, S. G.: J. Neuropath. & Exper. Path., to be published.

16. Smith, S. G.: Arch. Biochem. **20**:473, 1949.

17. Holler, J. W.: J. A. M. A. **131**:1186, 1946. Nicholson, W. M., and Branning, W. S.: ibid. **134**:1292, 1947. Stephens.[a,b]

18. Wiese, A. C.; Johnson, B. C., and Nevens, W. B.: Proc. Soc. Exper. Biol. & Med. **63**:521, 1946.

19. Flipse, R. J.; Huffman, C. F.; Duncan, C. W., and Thorp, F., Jr.: J. Animal Sc. **7**:525, 1948.

20. Wolbach, S. B., and Bessey, C. A.: Physiol. Rev. **22**:233, 1942.

21. Anderson, H. D.; Elvehjem, C. A., and Gonce, J. E.: Proc. Soc. Exper. Biol. & Med. **43**:750, 1939.

22. Speidel, C. C.: Am. J. Anat. **62**:179, 1938.

produced by, among other means, physical and chemical trauma, electricity, ischemia and starvation. Such muscle fibers, if not too seriously injured, are capable of complete regeneration. It is likewise known that similar muscle changes may appear in man in the course of an acute infectious disease.[22] It is apparent, therefore, that the lesion observed in the potassium-deficient dogs is in no sense pathognomonic.

The paralyzed dogs when untreated died within a period of eight to twenty-four hours after the onset of the progressive paralysis. This short survival period explains the minimal damage demonstrated histologically in the muscle. It is probable that only those fibers which were earliest or most severely damaged display this fact. The degree of paralysis obviously indicates a vastly more widespread damage of muscle, albeit of a less severe degree.

This short survival period after the onset of paralysis is also a factor to be considered in evaluating the results of the examination of the central nervous system. None of the myelin stains utilized are capable of revealing degeneration before several days after inception of the process. However, the fact that cresyl violet preparations fail to reveal any abnormalities in ganglion cells and that intravenous administration of potassium to a prostrate dog leads within minutes to recovery, even though temporary, impels to the conclusion that degeneration of neurons plays no role in the myopathy.

Since it is well known that single skeletal muscle fibers are capable of complete regeneration, it appears that when timely supplements of potassium are administered, either as the salt or in the form of brewers' yeast, little if any residuum of the paralysis should be seen. This hypothesis was tested in the case of 2 animals which were fed diet A until paralysis ensued. They were then fed diet E for fifteen and sixty-eight weeks, respectively. No evidence of the myopathy was seen in the muscle sections of either animal, though numerous muscle groups were examined, and no clinical evidence of residual paralysis was observed.

SUMMARY

We have studied 49 rats and 21 dogs subsisting on the same potassium-deficient diet, which lacked also certain members of the B complex. Our own work and the work of others have been confirmed in that both species ceased to grow and died if they were fed the diet continuously without supplement. The rats did not have paralysis or any very characteristic symptom complex during life. There was no reproduction on the deficient diet, but this could not be correlated specifically with lack of potassium. The deficiency led to necrosis and eventual fibrosis of the myocardium and dilatation of the renal loops of Henle. The skeletal muscles remained intact. The lesions of the heart and the kidneys can be correlated closely with the lack of potassium. There was

a change in the sebaceous glands of the tails, however, which did not correlate with potassium deficiency. In the comment the possibility that this was correlated with a B complex factor is suggested.

The dogs, on the other hand, regularly underwent a series of mild paralytic attacks followed by a very characteristic syndrome of progressive paralysis which, if untreated, led eventually to death due to respiratory failure. This syndrome is thought to be the animal analogue of periodic paralysis of man. A marked increase of the fluid exchange (water intake and urine output) occurred in the dogs, but this was not observed to any extent in the rats.

The myocardium and the renal loops of Henle remained intact in the dogs, but skeletal muscle revealed waxy degeneration. The central nervous systems of the 5 dogs examined (3 potassium-deficient and 2 yeast controls) disclosed no lesions which could be ascribed to potassium deficiency.

It has long been known that different animal species react differently to a given nutritional deficiency. The guinea pig, for instance, reacts to withdrawal of ascorbic acid (vitamin C) with the development of scurvy, while the rat is practically unaffected. On the other hand, the guinea pig is resistant to a lack of vitamin D, while the rat responds with the development of rickets in a comparatively short period, three to four weeks. In the present study we see that both the rat and the dog react to a lack of potassium in the diet but in different ways. In the rat no very specific clinical syndrome is observed, while in the dog a characteristic flaccid paralysis develops which is similar to, if not identical with, that produced in man under conditions leading to potassium deficit. It seems, then, that in studies designed to throw light on the human problem the dog is the animal of choice.

Merck & Company, Rahway, N. J., supplied pyridoxine and calcium pantothenate; Fleischmann Laboratories Standard Brands, Inc., New York, the brewers' yeast for our control diets; Lederle Laboratories, a grant in aid and supplies of choline, inositol, menadione and vi-delta® liquid concentrates of vitamins A and D and material with which the dogs were immunized against distemper, and the vermifuge used. This work was aided further by a grant from the Nutrition Foundation and a Duke University Research Grant.

EFFECT OF SUBCUTANEOUS AND INTRAORAL ADMINISTRATION OF CARBON TETRACHLORIDE ON THE LIVER OF THE RAT

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THAT carbon tetrachloride is toxic to the liver and the kidneys has been known for many years. Smillie and Pessoa [1] observed fatty changes in these organs in dogs after the use of small doses of the chemical. Docherty and Burgess [2] found similar changes in the livers of two condemned prisoners who ingested 5 cc. of the drug shortly before execution. Cameron and Karunaratne [3] injected it subcutaneously into rats. In their comprehensive report dealing with the acute and chronic effects of the chemical, they stated that they used as much as 0.1 cc. in 150 Gm. white male rats to produce the classic central necrosis of lobules of the liver and that the maximal effect was reached in seventy-two hours after the injection of a single dose. Winter [4] injected subcutaneously 0.5 cc. per hundred grams of body weight, and Sundareson [5] used as much as 0.3 cc. to produce changes the same as those described by Cameron and Karunaratne.[3]

In studying the protective action of certain chemicals on the liver of the rat, we desired a method whereby administration of a constant amount of carbon tetrachloride would be followed consistently by definite hepatic necrosis. We therefore adopted the technic described by Cameron and Karunaratne [3] but to our surprise were unable to reproduce with any degree of regularity the lesions described by them and others. Since we spent considerable time and effort in carrying out these preliminary studies, we thought it might be worth while to record our findings.

From the Department of Pathology, Jefferson Medical College.

1. Smillie, W. G., and Pessoa, S.: Internat. Health Board Bull. no. 7577, 1922, p. 1.
2. Docherty, J. F., and Burgess, E.: Brit. M. J. **2**:907, 1922.
3. Cameron, J. R., and Karunaratne, W. A. E.: J. Path. & Bact. **43**:1, 1936.
4. Winter, I. C.: J. Biol. Chem. **133**:123, 1940.
5. Sundareson, A.: J. Path. & Bact. **54**:289, 1942.

METHODS

Experiment 1.—Fourteen white male rats weighing between 150 and 165 Gm. were used. They were fed the stock house diet.[6] Chemically pure carbon tetrachloride[7] was injected subcutaneously. The doses used were 0.1, 0.2, 0.4, 2.0, 3.0, 4.0, 5.0 and 10 cc. The larger doses were injected into at least two different sites. All animals were killed by the use of illuminating gas on the third day except the one receiving 5 cc., which was killed on the fifth day.

Experiment 2.—Eighteen white male rats weighing between 150 and 300 Gm. were given subcutaneous injections of 0.1 to 15.0 cc. of chemically pure carbon tetrachloride.[8] These animals were fed the same diet as those of experiment 1. Because of the irregular and indecisive results obtained in experiment 1, the first 4 rats in this group, apparently in good health, were given second injections six days after initial doses of 0.1 to 1.0 cc., of amounts varying from 4.0 to 10.0 cc. They were killed two days later. The remaining rats received single doses of carbon tetrachloride as follows: 1 rat, 2.0 cc.; 1 rat, 4.0 cc.; 5 rats, 10.0 cc., and 7 rats, 15.0 cc. All animals receiving 15 cc. weighed between 200 and 300 Gm., while the remaining ones weighed from 150 to 165 Gm. All the 10.0 and 15.0 cc. doses were injected into two different regions of the back.

Experiment 3.—Twelve white male rats weighing between 250 and 300 Gm. were given chemically pure carbon tetrachloride[7] through a no. 10 rubber catheter which had been passed into the stomach by way of the oral cavity. These rats were fed the same diet and lived in the same environment (room, cage, temperature, water) as the animals used in the previous experiments. They were divided into two groups of 6 animals. Each of the animals in one group received 0.5 cc., and each in the other 1.0 cc., of carbon tetrachloride. They were killed one, two and three days after intubation.

RESULTS

For simplicity, the livers will be divided into three groups. Group 1 includes those which were microscopically normal. Group 2 encompasses the livers in which a range of reversible processes, such as parenchymatous and hydropic degeneration and fatty changes, was observed. Frequently the alterations seen in this group were accompanied by a moderate sprinkling of the area with chronic inflammatory cells. These changes appeared more often in the central areas of the lobules. In addition, the changes encountered by Brunschwig, Johnson and Nichols[9] are considered in this group. They consisted of centrally located foci of enlarged hepatic cells with clear, vacuolated cytoplasm and small nuclei. In the centers of these foci were shrunken hepatic cells with dense eosinophilic cytoplasm and a varying degree of lymphocytic infiltration. Group 3 includes the livers showing varying degrees of necrosis, central fibrosis of lobules, hemorrhages, reversible changes and slight lymphocytic infiltrations.

Experiment 1.—Grossly, the livers of all these rats were normal.

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6. This consisted of Purina Dog Chow Checkers.

7. This was obtained from Merck & Company, lot no. 41008.

8. This was C. P. Baker's analyzed, lot no. 33048, supplied by the J. T. Baker Chemical Company, Phillipsburg, N. J.

9. Brunschwig, A.; Johnson, C., and Nichols, S.: Proc. Soc. Exper. Biol. & Med. **60**:388, 1945.

Histologically, the livers from 4 rats given 0.1 cc., 0.1 cc., 0.2 cc. and 0.4 cc. of carbon tetrachloride, respectively, appeared normal and were placed in group 1. The livers from 8 rats given doses of 0.1 cc., 0.2 cc., 2.0 cc., 4.0 cc., and 10.0 cc. of carbon tetrachloride were placed in group 2. One rat showed the same degree of change but will not be included in group 2 because it died within twenty-four hours after receiving the injection. The liver of the rat which received 5.0 cc. of carbon tetrachloride in divided doses showed irreversible changes and was therefore placed in group 3.

Experiment 2.—Four animals in this group died within a few minutes to twenty-four hours after injection of carbon tetrachloride and will not be considered.

Grossly, 8 of the remaining 14 livers examined were normal. Of the other 6, the livers from the 2 rats given 10.0 cc. and the 4 rats given 15.0 cc. of carbon tetrachloride were approximately of normal size but varied from a drab, light brown to a darker, rusty brown. The surface and lobular markings were obscured, and the tissue was soft and somewhat friable.

Histologically, 1 liver from this group of 14 was normal and was therefore placed in group 1. It was removed from a rat which had received 10.0 cc. of carbon tetrachloride. Five livers were placed in group 2. The corresponding doses given these were 2.0 cc., 10.0 cc., 15.0 cc., 15.0 cc., and 6.3 cc. (the last in divided doses). Eight livers were put in group 3. These were from rats given divided doses of 8.5 cc. and 11.0 cc. and single doses of 10.0 cc., 10.0 cc., 15.0 cc., 15.0 cc. 15.0 cc. and 15.0 cc. of carbon tetrachloride.

Experiment 3.—One of the rats in this group that received 1.0 cc. of carbon tetrachloride was found dead in less than twenty-four hours and so will be omitted.

Grossly, all of the remaining 11 livers were quite similar and essentially normal in size. They were soft and friable and tore when slight to moderate pressure was exerted, so that removing them intact from the abdominal cavities was practically impossible. Some were dark, reddish-brown; the remainder were yellowish tan. The surface and lobular markings were uniformly obscured.

Histologically, 10 of the rats that received carbon tetrachloride intraorally (6 of them 0.5 cc. and 4 of them 1.0 cc.) showed central lobular necrosis of the liver and were placed in group 3. In addition, an animal which was given 1.0 cc. of carbon tetrachloride intraorally and which was killed twenty-four hours later had degenerative and necrotic changes diffusely throughout the lobules. Accordingly, the liver of this rat was also placed in group 3.

COMMENT

The inconsistencies of the results following the subcutaneous administration of carbon tetrachloride appear significant. Apparently, other investigators have had similar difficulties, although this is not mentioned in the literature. Dr. Paul György, in a personal communication, stated that he has discarded the use of subcutaneous injections to produce hepatic necrosis because of the irregularities encountered. The following statement is found in a letter from the Safety Research Institute, Incorporated: "According to one of our informants, 'one may observe rather surprising differences in results among separate lots of animals from one source, all subjected to the same treatment in succession.'" Brunschwig, Johnson and Nichols [9] mentioned that in their experiment 2 of 15 rats showed "lesions of limited size."

In an effort to explain these findings, we wondered whether the diet was affording protection. Calder [10] found that a diet deficient in either choline or some unidentifiable thermolabile vitamin B substance afforded protection against hepatic damage following subcutaneous injection of carbon tetrachloride in rats. The livers were not protected if the diet contained adequate amounts of both these compounds or if the diet was deficient in both choline and this unidentifiable vitamin B substance. He also noted that the animals maintained on diets deficient in either or both of these substances failed to gain weight, whereas there was a steady increase in weight in the rats fed an adequate diet. At the time of acquisition our rats averaged 150 Gm., and at the end of the experiments the average weight had increased to 310 Gm. The animals were fed a diet which apparently contains adequate amounts of choline and vitamin B substances.[6] Furthermore, this is more or less a standard diet, since other experimenters [11] have used it to feed rats with impunity. This combination of circumstances leads us to conclude that the diet had little or no effect on the final results.

In a series of communications with the Safety Research Institute, Incorporated,[12] we learned that carbon tetrachloride produced in 1936 contained as much as 0.5 per cent organic sulfides, particularly carbon disulfide. Postulating that this substance, alone or in combination with carbon tetrachloride, might have produced the changes described by Cameron and Karunaratne,[5] we gave 4 rats 0.1 to 0.4 cc. of carbon disulfide, 2 rats 0.2 cc. of carbon disulfide and 2 rats 0.2 cc. of carbon disulfide, along with 3.0 and 4.0 cc. of carbon tetrachloride, by subcutaneous injection. All of these animals were killed three days later. Their livers revealed microscopic patterns varying from normal to some characterized by various reversible degenerative changes. None showed more than shrunken hepatic cells surrounded by a zone of enlarged, vacuolated cells and interspersed with a scattering of lymphocytes in the central areas of the lobules.

The skin at the site of the injection was examined routinely, and only minimal changes were noted. The larger doses produced areas of edema with little or no necrosis and slight inflammatory cell infiltration. Although the edema fluid was not tested chemically for the presence of residual carbon tetrachloride or carbon disulfide, the characteristic odor of each was absent at the time of examination. Sundareson [9] reported similar findings.

10. Calder, R. M.: J. Path. & Bact. **54**:355, 1942.

11. (*a*) Fitzburgh, O. G.: Proc. Soc. Exper. Biol. & Med. **40**:11, 1939. (*b*) Neale, R. C., and Winter, H. C.: J. Pharmacol. & Exper. Therap. **62**:127, 1938.

12. Hutchins, B. C.: Personal communication to the authors.

Consistent hepatic necrosis was found in the 11 animals of the last experiment, in which carbon tetrachloride was administered orally. Incidentally, this carbon tetrachloride was from the same bottle as that used in the subcutaneous injections of experiment 1. The changes were most pronounced in the centers of the lobules, and the results are similar, therefore, to those reported by others.[13] The rats receiving the drug orally were given doses of from 1.6 to 3.3 cc. per kilogram of body weight, whereas those receiving it subcutaneously were given as much as 71.4 cc. per kilogram of body weight. The actual passing of a catheter into a rat's stomach is simple, requiring little time or practice.

Another apparently consistent and reliable way to produce hepatic necrosis is by administering carbon tetrachloride by inhalation.[14] We have had no experience with that method.

SUMMARY

Carbon tetrachloride when subcutaneously injected into rats in doses as high as 15 cc. produced hepatic necrosis in 8 of 27 animals. The amounts given to 7 of these were between 10 and 15 cc. Carbon tetrachloride administered by way of the alimentary canal in amounts of 0.5 to 1.0 cc. produced necrosis of the liver in all 11 animals used.

It is concluded, therefore, that carbon tetrachloride should be administered by way of the alimentary tract rather than subcutaneously in order to produce hepatic necrosis consistently.

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13. Lacquet, A. M.: Arch. Path. **14**:164, 1932. Boericke, G. W., and Wastl, H.: Hahneman. Monthly **78**:24, 1934. Fitzburgh.[11a]

14. Forbes, J. C., and Neale, R. C.: Proc. Soc. Exper. Biol. & Med. **34**:319, 1936. Bollman, J. L.: Collect. Papers Mayo Clin. & Mayo Found. **37**:814, 1945. Machella, T. E., and Higgins, G. M.: Am. J. M. Sc. **204**:194, 1942. Wakim, K. G., and Mann, F. C.: Arch. Path. **33**:198, 1942. Neale and Winter.[11b]

General Reviews

CHORIOCARCINOMA

A General Review, with an Analysis of Five Hundred and Sixteen Cases

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(Continued from p. 104)

ONSET, BEHAVIOR AND SPREAD OF CHORIOMA

THE FACT that there are no distinctive histologic criteria of canceration in choriocarcinoma means that, given an undoubted trophoblastic hyperplasia, one has no way of assessing the degree of hyperplasia or the rate of growth except in terms of how large it is or how far it has spread.

The rarity of choriocarcinoma means that a wide review can be based only on reported cases, which tend to be of unusual interest because they deviate from the mode. It is impossible to estimate the frequency of cases such as these relative to that of the ordinary straightforward type.

TROPHOBLAST OCCURRING WITHIN THE TISSUES

The Trophoblastic Node.—Just as tuberculosis has a characteristic lesion, the tubercle, so chorioma has a characteristic lesion, a dark red or purple nodule, already described as a trophoblastic node. Microscopically, it is a mass of blood with a rim of trophoblastic tissue. This rim may form a very small part of the total volume of the nodule, and it is not surprising that sometimes trophoblastic cells cannot be found, as, for example, in the vaginal nodule in a case described by Mandelstamm.[87] It is rare for a metastasizing neoplasm, even hypernephroma, to simulate this hemorrhagic appearance.

The hemorrhagic nature of the node is usually attributed to a retention of the alleged physiologic capacity of the trophoblastic cells for invading blood vessels. This explanation is inadequate. Most cancers invade blood vessels—and indeed must do so if they are to produce blood-borne metastases. A choriocarcinoma of the liver is accompanied by a large intratumoral extravasation of blood, and an equally extensive secondary

From the Royal College of Physicians' Laboratory.

87. Mandelstamm, A.: Arch. f. Gynäk. **160**:239, 1936.

rectal carcinoma of the liver is not. The explanation cannot be that the blood channels were eroded in the one case and not in the other. The difference must lie in some local disturbance of bleeding time or coagulability associated with the presence of trophoblastic cells. It may be that this is connected with the physiologic absence of clotting of blood at the placental site—possibly a function of the trophoblast. It is even possible that it is related to the absence of clotting in menstrual blood.

Hypothetic ferments and lysins have been invoked to explain the behavior of trophoblast and chorioma. Evidence that fetal cells can produce effects at a distance, presumably by a chemical agency, is provided by several observations—hemorrhage involving the posterior chamber of a mouse's eye the anterior chamber of which contains an implanted ovum (Fawcett, Wislocki and Waldo [59]); epithelial proliferation of the endometrium of the macaque opposite the abembryonic pole of the ovum before actual contact of the tissues (Wislocki and Streeter [256]) and the finding in a human uterus of an area of congestion and hemorrhage opposite and close to, but not in contact with, an implanting ovum (Hertig and Rock [108]). Still the existence of such agencies can be deduced and defined only from the appearances found in association with fetal cells. It may be remarked here that no one has yet shown that cancer cells in general have any cytolytic properties.

One concept which runs through the whole history of the study of choriocarcinoma is based on analogy with the normal embedding trophoblast, the "invasiveness" of which is assumed to have first a histiotropic and then a hemotropic phase. Similarly it is assumed that cancerous trophoblast grows and spreads preferentially and more easily in blood than in fixed tissues and that when it has to grow in fixed tissues it stimulates a defensive reaction whose physiologic analogue is the layer of Nitabuch. This is fibrinoid and coagulative and is assumed to act by denying nutrition to, and by physically compressing, the trophoblast. This is, of course, merely a restatement in teleologic form of what can be seen under the microscope, and fifty years of study and experiment has not made it any more.

Outside the uterus a node such as we have described is completely characteristic of chorioma. Inside the uterus it is also characteristic of chorioma except for the "atypical" variety of Marchand (syncytioma, syncytial endometritis of other writers), though even here there is extravasation of blood. The trophoblastic node is not only a manifestation of the frankly cancerous, metastasizing trophoblast of choriocarcinoma; it is also found, apparently identical, in the less destructive hydatidiform mole ("chorio-adenoma" of Ewing). It is found, still discrete and localized, in many uteri removed from patients who subsequently recover. Trophoblast proliferating in the lumen of the uterine cavity cannot, of course, produce these nodes. In metastasizing choriocarcinoma

one may find little islets of trophoblast, usually intravascular, which have not formed such nodes, presumably because there has not been time for them to develop. In general, however, it may be said that the trophoblastic node is the manifestation of trophoblast occurring in a tissue. The morbid anatomy of chorioma and choriocarcinoma is the aggregation of such nodes varying in distribution by site and by time.

Suddenness of Onset of the Trophoblastic Node.—The vagina is the only site where the behavior of such nodes can be observed from day to day. In most instances, when a patient has a vaginal nodule it was already present at the time of the first examination. Sometimes, however, it has happened that there has been daily examination of the vagina for one reason or another, and that suddenly, overnight, a typical purplish nodule has appeared. Such a happening was recorded by Brenner.[88] Hitschmann and Cristofoletti[89] and Reeb[90] reported two trophoblastic nodules arising within two days and four days, respectively. For this the most likely explanation is a sudden hemorrhage. It seems probable that this is the cause of both the suddenness of onset, when it occurs, and the actual swelling of many of these nodes.

Regressibility of the Trophoblastic Node.—In a small proportion of cases of what would otherwise be called choriocarcinoma regression occurs. If "choriocarcinoma" is taken to mean isolated nodes occurring in the uterus, the regression rate is possibly much higher.

For this regressibility of individual nodes there is a good deal of evidence, especially in the vagina and to a lesser degree in the lung (see page 223).

In the uterus the evidence is necessarily circumstantial. The symptoms and clinical signs of an intramural trophoblastic node are not clear-cut and certainly do not distinguish those nodes which will progress to metastasis in spite of hysterectomy, those which will be cured by hysterectomy and those which will regress spontaneously. Since it is only when a trophoblastic node is suspected that the uterus is removed, there is no definite evidence how often such nodes occur unsuspected in the uterine wall, whether or not they frequently cause frank symptoms, or how often they regress spontaneously. A remark of Novak's[91] has relevance here. In discussing the after-supervision of a patient who has had a hydatidiform mole he stated:

> Should recurrence of bleeding or biological tests lead to the suspicion of malignancy, most gynecologists will undoubtedly consider hysterectomy advisable.

88. Brenner, M.: Monatschr. f. Geburtsch. u. Gynäk. **27**:574 and 713, 1908.

89. Hitschmann, F., and Cristofoletti, R.: Wien. klin. Wchnschr. **24**:655 and 705, 1911.

90. Reeb, M.: Arch. f. Gynäk. **71**:379, 1904.

91. Novak, E.: Textbook of Gynecology, ed. 3, Baltimore, Williams & Wilkins Company, 1948, p. 690.

There can be no criticism of such a policy, but there is no doubt that in a large proportion of cases *subsequent* examination of the removed uterus will show merely a residuum of hydatidiform tissue instead of chorionepithelioma.

In addition there have been occasions when a patient has been considered as having choriocarcinoma, has been advised to undergo hysterectomy, has refused and has made a complete recovery. Examples of this chain of events have been recorded by Fleischmann,[92a] Pick,[92b] Hofmann[93] and Hertig and Sheldon.[94]

There are also cases in which a patient has many of the indications of choriocarcinoma but in which the gynecologist, with a natural reluctance to remove the uterus of a young woman, worries and delays and waits for reports of Aschheim-Zondek tests and other opinions. Often in the end his patient is rapidly improving before he has been able to decide definitely. As in the other circumstances that have been mentioned, the question here is: What would the uterus in a case such as this have shown if it had been removed by another gynecologist with slightly different, though equally reasonable, evaluations of the clinical data? Clearly, so far as the uterus is concerned we can get little further than speculation as to how often trophoblastic nodes are present and regress.

EXTRAUTERINE SITES OF CHORIOMA

Primary gestational chorioma is, of course, by definition restricted to the usual gestational sites. Since the conditions of nidation are different in the uterus and in the fallopian tube, the ovary and the abdominal cavity, an incidence of choriocarcinoma disproportionate to the incidence of pregnancy at these sites would be of great significance.

In the Tube.—Novak[35] gave the proportion of tubal to uterine gestation as 1 : 300. Mathieu[7] calculated that up to 1939 some 1,500 or more cases of choriocarcinoma had been reported in the literature. The number of cases of tubal choriocarcinoma reported up to the end of 1939 was at least 47 (see page 227). This gives a proportion of tubal to uterine choriocarcinoma of approximately 1 : 30. Whether this is to be explained

92. See Teacher's[1] list of annotations, pp. 170-199: (*a*) case 28; (*b*) case 135; (*c*) case 41; (*d*) cases 8 and 119; (*e*) case 164; (*f*) case 178; (*g*) case 183; (*h*) case 56; (*i*) case 62; (*j*) case 74; (*k*) cases 6, 38, 108, 116, 138 and 162; (*l*) case 33; (*m*) case 37; (*n*) case 148; (*o*) cases 151 and 152; (*p*) case 186; (*q*) case 52; (*r*) case 87; (*s*) case 155; (*t*) case 180; (*u*) case 17; (*v*) case 67; (*w*) case 97; (*x*) case 156; (*y*) case 189; (*z*) case 162; (*a'*) case 117; (*b'*) case 136; (*c'*) case 49; (*d'*) case 63; (*e'*) case 91; (*f'*) cases 115 and 117; (*g'*) case 8; (*h'*) case 24; (*i'*) case 77; (*j'*) case 78; (*k'*) case 129; (*l'*) case 19; (*m'*) case 69; (*n'*) case 2; (*o'*) case 3; (*p'*) case 36; (*q'*) case 120; (*r'*) case 142; (*s'*) case 159.

93. Hofmann, H.: Monatschr. f. Geburtsch. u. Gynäk. **93**:311, 1933.

94. Hertig, A. T., and Sheldon, W. H.: Am. J. Obst. & Gynec. **53**:1, 1947.

on the ground that every case of tubal choriocarcinoma, and only 1 of every 10 cases of uterine choriocarcinoma, is reported can only be a matter of opinion. Our opinion would be that the evidence as it stands is not sufficient to prove that there is a disproportionate difference in incidence of choriocarcinoma in the tube and in the uterus.

In the Ovary.—On the rare occasions when choriocarcinoma is found in the ovary it is difficult to decide whether the tumor has arisen from teratoma or is secondary to an undiscovered or regressed primary tumor of the uterus or the tube. The problem has been discussed by de Zalka.[91] In any case the incidence of ovarian choriocarcinoma, gestational or not, is so small that it is unlikely that the incidence of gestational ovarian choriocarcinoma is more than would be expected from the frequency of ovarian pregnancy.

In the Abdomen.—So far as we know, no case of choriocarcinoma resulting from an abdominal pregnancy has been reported, and none is mentioned by Gardner and Middlebrook[95] in their review of 258 cases of abdominal pregnancy. (The nearest approach is the case described by Lazarus and Schifrin[96] in which multiple chorionic implants were present in the peritoneal cavity.) This would again suggest that the incidence of choriocarcinoma relative to the incidence of pregnancy does not vary with the site of gestation.

INTRAUTERINE SITES OF CHORIOMA

Davis and Brunschwig[97] have described the case of a patient who had been regarded clinically as having a central placenta previa. A living 6 month fetus was delivered. The normal placental site was found high in the fundus, the placenta being easily removed, while the whole of the lower segment was occupied by a friable mass diagnosed as choriocarcinoma.

A few examples of choriocarcinoma restricted to the uterine cervix have been reported—by von Guérard,[92c] Bourne,[98] Schimmel,[99] Meyer [43] and Rubin.[100] Apart from the case of Schimmel, in which there was no follow-up, the patients recovered.

Aside from these few exceptions it seems that choriocarcinoma is confined to the placental site, and otherwise has no selective localization within the uterus.

95. Gardner, A. R., and Middlebrook, G.: Am. J. Surg. **66**:161, 1944.
96. Lazarus, J. A., and Schifrin, A.: Ann. Surg. **115**:93, 1942.
97. Davis, M. E., and Brunschwig, A.: Am. J. Obst. & Gynec. **31**:987, 1936.
98. Bourne, A. W.: Proc. Roy. Soc. Med. **18**:89, 1924-1925.
99. Schimmel, H.: Zentralbl. f. Gynäk. **49**:2469, 1925.
100. Rubin, I. C.: Am. J. Obst. & Gynec. **41**:1063, 1941.

TIME FACTOR IN GESTATIONAL CHORIOCARCINOMA

When Does Canceration Start?—A gestational choriocarcinoma can certainly start only after conception. Apart from this the only evidence for assessing its date of starting is the clinical appearance and behavior of the tumor. Since choriocarcinoma has been found after only one missed menstrual period, and since it may occur, rapidly growing, after a full time and otherwise normal pregnancy, it may be said to arise at any time during the life of the trophoblast. The further question of whether the tumor has a selective frequency at any given time after conception is complicated by the difficulties that no one knows how often choriocarcinoma itself causes abortion, how often refertilizations and abortions occur after a pregnancy, and how long the tumor has taken to reach the stage of clinical recognition.

Figures given earlier show that the mean survival time after hysterectomy is four months. One can only guess, but it would be reasonable to think that the time from the onset of cancer to its clinical manifestations would not be longer than the time between clinical manifestations and generalized metastasis. Although there are a few reported cases suggesting the contrary, it is unlikely that a pregnancy could continue for long in the presence of an active intrauterine cancer. Certainly, assuming that the proportion of cases in which choriocarcinoma occurs after a normal full time pregnancy is one fourth to one third of the total number of cases of choriocarcinoma and allowing, say, one to three months for the tumor to manifest itself clinically, one might legitimately deduce that the incidence of cancerous transformation is rather evenly distributed over the life of the trophoblast. It might even be that it is selectively frequent in abnormally retained trophoblast. That is, its origin is related not to a given time after conception, nor presumably to a direct chain of causes starting then, but to the presence of trophoblast itself.

Trophoblastic proliferation and "invasiveness" are maximal during the first two months of pregnancy. This is a phenomenon of remarkable constancy in time and degree. If the cause of choriocarcinoma were closely related to either of these factors, one would expect the tumor to start soon after conception, or at any rate at a fairly constant interval of time thereafter, and possibly also to manifest itself clinically at a fairly constant interval of time after conception. There is, of course, no such constancy.

The even distribution of the onsets of choriocarcinoma throughout the life of the trophoblast suggests a maternal influence in the causation of choriocarcinoma.

When Does the Tumor Appear?—The diagnosis of choriocarcinoma is not usually made when the patient is first seen, as with most tumors,

but only after a period of increasing clinical suspicion. Consequently, the only sharply defined date line which can be used for the time of diagnosis is the date line of hysterectomy.

From the figures given earlier it will be seen that when symptoms develop so rapidly that choriocarcinoma is diagnosed only when the lesion is inoperable (often because of metastases) or at death, the intervals of time after cessation of the last pregnancy are five months and 6.1 months, respectively. In many cases, however, the development of the disease is sufficiently slow to allow a hopeful result from hysterectomy, which is carried out also at about five to eight months. In other words, all types of choriocarcinoma, no matter what the rate of growth of the tumor, are diagnosed, maybe hopelessly, at a mean time of about 6.0 months after the cessation of pregnancy. It is to be hoped that with better management in doubtful cases diagnosis will be earlier, with a consequent increase in the proportion of cases in which hysterectomy promises a favorable outcome. It is doubtful, however, whether even with modern methods of diagnosis earlier diagnosis will not mean an increase in less accurate diagnosis.

Time of Death.—Our figures show that in undiagnosed cases death occurs at a mean time of six months after the cessation of pregnancy, and, if a diagnosis is made before death, two to four months after the diagnosis has been made. In other words, choriocarcinoma is an acutely dangerous tumor, probably the most rapidly killing tumor in the whole field of pathology.

Long Delay in Development or Recurrence of Choriocarcinoma.—The claim that metastases have appeared long after the discovery of the primary growth, and the claim that the primary growth has appeared long after the pregnancy concerned, are two aspects of the problem which, while distinct, are related and can be considered together.

The only chain of events to be accepted as proof positive of delayed recurrence is the appearance of a primary choriocarcinoma, diagnosed and treated as such at the time, and then, not sooner than two years thereafter, the appearance of secondary tumors with the structure and behavior of choriocarcinoma. Usually this would mean a primary choriocarcinoma diagnosed after removal of the uterus. The diagnosis of a primary choriocarcinoma in retrospect is much less satisfactory.

If this delay of recurrence is relatively frequent in cases of choriocarcinoma, it would be expected that some at least of the reported claims would satisfy the absolute criteria just laid down. There is the case reported by Cary [101] in which hysterectomy was carried out four months after an abortion, and a parametrial mass was found. The patient died thirty-eight months later with generalized metastases, diagnosed as

101. Cary, E.: Surg., Gynec. & Obst. **16**:362, 1913.

choriocarcinoma. There is, however, no specific mention that the original parametrial mass was diagnosed as choriocarcinoma at the time of removal. Besides this, our own series includes the case of a woman of 55 years on whom hysterectomy was performed for undoubted chorioma. She died five years later with "radiological and clinical evidence of metastases in brain and spinal cord"—almost certainly, however, from an advanced mammary carcinoma removed eighteen months after the removal of the uterus.

TABLE 5.—*Reported Cases of Latency of Development of Choriocarcinoma*

Case	Author	Age of Patient	Type of Preceding Pregnancy	Interval Between Last Pregnancy and Diagnosis	Result
1	Sandberg [108]	50	Abortion	5⅝ yr.; tumor diagnosed at operation	Alive for at least 3 months after hysterectomy and excision of vaginal nodule
2	Caturani, M.: Am. J. Obst. 63:614, 1911	51	Hydatidiform mole	4¾ yr.; tumor diagnosed at operation	Died 3 months after curettage; clinical evidence of metastases in lung and brain
3	Cary [101]	37	Abortion	3½ yr.; tumor diagnosed at death	Died; multiple tumor metastases *
4	Outerbridge, G. W.: Am. J. Obst. 72:958 and 1060, 1915	41	Abortion	5½ yr.; tumor diagnosed at operation	Alive at least 5 months after excision of vaginal mass
5	Polano, O.: Ztschr. f. Geburtsh. u. Gynäk. 75:149, 1914	58	Normal	10 yr.; tumor diagnosed at operation	Died 10 months after excision of vaginal tumors
6	Geist, S. H.: Am. J. Obst. 72:472, 1915	46	Normal	3¼ yr.; tumor diagnosed at operation	Alive and apparently cured by hysterectomy
7	Adachi [103]	45	Abortion	2 yr.; tumor diagnosed at death	Died from "exhaustion"; no mention of metastases
8	Taylor, H. C.: Am. J. Obst. 75:671, 1917	32	Abortion	3¼ yr.; tumor diagnosed at operation	Died 9 months after hysterectomy and excision of vaginal nodules; clinical evidence of metastases in lung and brain
9	Schwarzer: Arch. f. Gynäk. 112:212, 1920	39	Normal	2¼ yr.; tumor diagnosed at operation	Died 9 months after hysterectomy; clinical evidence of metastases in brain
10	Von Ralsz, D.: Zentralbl. f. Gynäk. 54:2867, 1930	34	Hydatidiform mole	5 yr.; tumor diagnosed at operation	Died 1 month after hysterectomy; no autopsy
11	Brown, A. F.; Snodgrass, W., and Pratt, O. B.: Am. J. Cancer 38:564, 1940	32	Hydatidiform mole	9 yr.; tumor diagnosed at death	Died; multiple tumor metastases †
12	Docherty, M. B., and Craig, W. M.: Am. J. Obst. & Gynec. 44:497, 1942	29	Hydatidiform mole	4 yr.; tumor diagnosed at operation	Died 6 weeks after craniotomy; no autopsy; no clinical evidence of other metastases ‡
13	Maier and Taylor [140]	32	Hydatidiform mole	3½ yr.; tumor diagnosed at operation	Alive for at least 3 years after lobectomy and hysterectomy §
14	Case 15, our own series.....	55	Abortion	4 yr.; tumor diagnosed at operation	Died 5 years after hysterectomy and 3¼ years after mastectomy; clinical evidence of metastases in brain and spinal cord ‖

* Hysterectomy was carried out four months after the abortion. A small mass was found in the parovarium. There is no specific mention of its having been diagnosed as choriocarcinoma at that time.

† Hysterectomy was carried out for dysmenorrhea two years after passage of a hydatidiform mole. There was no evidence of cancer of the uterus.

‡ Hysterectomy was carried out soon after passage of a hydatidiform mole. The mole was reported as showing no penetration of the uterine wall. The patient was admitted four years later with a brain tumor, which was removed and diagnosed as choriocarcinoma.

§ No tumor was found in the uterus, which was removed after lobectomy for a tumor of the lung, which was diagnosed as choriocarcinoma.

‖ The menopause occurred three years before diagnosis. Hysterectomy was done. Advanced mammary carcinoma was diagnosed and the tumor removed one and a half years after hysterectomy.

Apart from these 2 examples, we have not seen reports of any cases which would satisfy these criteria. Neither of these examples can be considered sufficiently convincing to establish the principle that delayed recurrence of choriocarcinoma occurs at all; and far less, that it is relatively common. It must, in fact, be extremely rare as compared with, for example, melanocarcinoma, carcinoma of the breast and many other tumors the frequency of the delayed recurrence of which is undoubted.

An example of latency of development of the primary tumor should satisfy the criterion that conception had been impossible during the interval between the pregnancy claimed to be causative and the development of the disease. Hitschmann and Cristofoletti [69] said they would prefer to postulate a fresh pregnancy, even after the menopause, rather than accept long latency of the development of a choriocarcinoma. There is here the difficulty that the risk of choriocarcinoma developing soon is some two to four thousand times greater after a hydatidiform mole than after a normal pregnancy. If, therefore, a hydatidiform mole, diagnosed at the time, occurred two or more years before the appearance of the choriocarcinoma, with no frank intervening pregnancy, the probability that the hydatidiform mole was the "causative" pregnancy is at least as high as the probability that a fresh pregnancy occurred unrecognized or unadmitted.

Krösing [102] described an example of delayed development of choriocarcinoma and gave a list of 20 other such cases. In table 5 some further examples are given. All the patients were said to have suffered from choriocarcinoma, and the descriptions are quite compatible with this diagnosis. It would be expected, however, that quite apart from any question of intervening pregnancy some or many of these cases would have been straightforward, typical examples of choriocarcinoma going on to produce disseminated proved metastases and death. In only 2 cases was this so—cases 2 and 8 (though even in these the evidence of metastasis was clinical only). Case 9 could also be regarded as typical, but it is difficult from the report of the case to make out the exact intervals of time. It is also to be noted that in only 1 case did the choriocarcinoma follow a hydatidiform mole, and, as mentioned earlier, only the strong selective action of a precedent hydatidiform mole would compensate for the inherent probability that "delayed" choriocarcinoma is due to an unrecognized and intervening pregnancy if that was possible.

There are, however, 3 cases in table 5 which satisfy the rigid criterion that intervening pregnancy should have been impossible—cases 3, 11 and 12. Krösing's own case also satisfied this criterion (though the

102. Krösing, E.: Arch. f. Gynäk. **88**:469, 1909.

fact that bilateral oophorectomy was carried out three and one-quarter years before hysterectomy and discovery of the choriocarcinoma can only be inferred from the data; there is no specific mention of it in the report of the case).

There are also several examples in Krösing's series of choriocarcinoma developing several years after the menopause, and there is our own case 15 (case 14 in table 5) in which choriocarcinoma developed as an undoubted intramyometrial trophoblastic node at age 55, three years after the menopause. It may be that postmenopausal women can conceive, though it is rare for them to achieve a normal full time pregnancy. Further, in the figures from which we estimated the interval of time between cessation of pregnancy and (*a*) diagnosis, (*b*) death, although the mean interval between cessation of pregnancy and diagnosis was about six months, several cases in fact had an interval of between one and two years. One can well imagine that over hundreds of cases there would be an occasional case with an interval of more than two years. It appears, therefore, that delay of the development of choriocarcinoma is not infrequent but that delay of recurrence is rare or does not occur. Two inferences can be derived:

1. That the influence that makes retained trophoblast flare up into cancer is probably maternal.

It is suggestive that many of the patients showing what must be accepted as evidence of delay of the onset of choriocarcinoma are of relatively high age—in the 40's and 50's, the "cancer age." In fact, in only 7 of Krösing's 21 examples was the patient below the age of 45, and each of these was not very convincing.

2. That choriocarcinoma is always a relatively acute disease and in this way differs from nearly every other type of tumor.

In this context it may be mentioned that Ries [103] reported observing chorionic villi in the uterus eighteen years after the last pregnancy, regarded as residual from that pregnancy. Since the woman's age was only 45 and since the villi had no covering of trophoblast and were only doubtfully villi at best, this report is quite unacceptable. It is, however, still recorded in textbooks.

TROPHOBLASTIC TISSUE REMAINING, ABNORMAL, WITHIN THE UTERUS

Simple "Retained Products of Conception".—In the great majority of cases one may presume that any residual trophoblastic tissue degenerates and disappears. Even in cases in which curettage is necessary, it is rare for the question of abnormal trophoblastic proliferation to arise, and nearly always such trophoblast as remains is no more than would be expected with a normal villus.

103. Ries, E.: Am. J. Obst. **67**:433, 1913.

Placental Polyp.—Occasionally one or more villi are able to maintain themselves as an apparently healthy organized unit. There is no visible trophoblastic hyperplasia or visible cellular degeneration. Such a placental polyp rarely shows undue trophoblastic hyperplasia and can usually be sharply separated from chorioma.

Hydatidiform Mole.—Many areas of hydatidiform mole may show villi with little or no trophoblastic covering, but it is generally agreed that some villi somewhere will show undue trophoblastic proliferation. To the extent that a hydatidiform mole shows this proliferation it is a chorioma. Many "simple" hydatidiform moles cause little trouble and disappear without sequelae after curettage and sometimes after spontaneous extrusion.

Choriocarcinoma is disproportionately preceded in time by hydatidiform mole, and in that sense hydatidiform mole is precancerous. The relation between the epithelial hyperplasias in the two diseases can be summarized only in the bleak fact that the choriomatous proliferation of choriocarcinoma is preceded in time by the choriomatous proliferation of hydatidiform mole in 30 to 50 per cent of cases.

Our figures show clearly that in the fifth decade there is a greatly increased incidence of choriocarcinoma per number of maternities. It is well known and accepted that there is a similar increased frequency of hydatidiform mole. Whether in fact these two increases run parallel, so that the risk of cancer following hydatidiform mole is not increased with age, we do not know, but we have the impression that there is no such increased risk. This does not mean, of course, that, although the risk of sequent choriocarcinoma is the same, the objections to radical preventive measures are not much less in an older woman than in a younger. Certainly we would not accept the contention that 1 of every 2 hydatidiform moles occurring in the 40 to 50 age group will become cancerous.

Invasive Hydatidiform Mole.—So-called invasive hydatidiform moles form a class in which the proliferative mass is more deeply rooted and seems to excavate for itself a wide hollow in the uterine wall, which becomes greatly thinned at this area and may be perforated. Such moles were studied and described in detail by Proust and Bender.[104] So far as can be inferred from reported cases invasive hydatidiform mole does not differ from simple hydatidiform mole except in this erosive character, neither in microscopic appearance nor in symptoms nor in the risk of subsequent choriocarcinoma. In fact, no case can be put into this category until the uterus has been palpated or examined visually: and this category is composed entirely of such cases.

104. Proust, R., and Bender, X.: Ann. de gynéc. et d'obst. **70**:231, 1913.

From the series of 493 cases recorded in the literature 47 cases were extracted, all of which can be placed in this category of invasive hydatidiform mole. In many of these the author himself defined the lesion as "malignant" or "invasive" hydatidiform mole, at the same time describing it as a form of choriocarcinoma. In the others the authors did not particularize the moles as invasive, but if there was a description of hydatidiform villi occurring deep within the uterine wall, the case was included in this list. In 3 of these 47 cases there was no follow-up. Twenty-seven of the remaining cases were nonfatal. Seventeen were fatal: Of the patients who died, 2 had choriocarcinoma with multiple metastases at death [92d]; 1 died of probable choriocarcinoma with vaginal metastasis [92e]; 1 died of possible choriocarcinoma (Bland [105]); 1 died six months later from an unknown cause [92f]; 1 died with a ruptured uterus [92g]; 1 died of external hemorrhage [92h]; 3 died of intraperitoneal hemorrhage (case 62 in Teacher's series [92i]; Hammerschlag,[106] case 5; Rosenbloom [107]); 1 died of peritonitis [92j]; 6 died postoperatively (all in Teacher's series [92k]).

It must be emphasized that all these cases of invasive hydatidiform mole were published primarily as cases of choriocarcinoma. How many hydatidiform moles have occurred which were more or less invasive but which were not published as examples of choriocarcinoma can only be guessed.

In only 2, or perhaps 3, of these 47 cases did a mole diagnosed as choriocarcinoma kill by metastatic spread. From these figures it is a safe inference that the risk of choriocarcinoma developing is no greater after the diagnosis of invasive hydatidiform mole than after that of simple hydatidiform mole. A gynecologist who diagnoses invasive hydatidiform mole should therefore not allow his treatment to be affected by the fear of subsequent cancer any more than with hydatidiform mole of any type.

Twenty-seven cases were nonfatal. It is interesting to speculate what the patients were saved from by hysterectomy. From choriocarcinoma? Ewing [6a] stated that he had, in 1940, yet to be convinced that a single patient of this kind had been cured, and that is also our impression. From fatal hemorrhage, rupture of the uterus, peritonitis? Six patients died of these causes, all but 1, it may be noted, before 1905; and a further 6 patients died more or less directly of the operation itself. In any given case the surgeon himself will assess the risk that such complications may occur, and also his chances of dealing successfully with them if they do. The evidence suggests that the risk of complications occurring is not great and that if they do occur they can be dealt with successfully.

105. Bland, P. B.: Am. J. Obst. & Gynec. **13**:189, 1927.
106. Hammerschlag: Ztschr. f. Geburtsh. u. Gynäk. **52**:209, 1904.
107. Rosenbloom, D.: Am. J. Obst. & Gynec. **43**:133, 1942.

The situation may be summarized by saying that if a hydatidiform mole is diagnosed as being "invasive," the risk of choriocarcinoma is no greater than with any hydatidiform mole and is probably not diminished by hysterectomy; that the risk of hemorrhage, rupture, etc., is not great; that if such catastrophes develop, they are treatable, and that the basis of the treatment of the mole should be conservatism. In one of his classic papers Marchand [40c] emphasized the difficulty of diagnosing cancer in a hydatidiform mole: "It warns us to be cautious about too radical a procedure, for here we must not despise too much the vis medicatrix naturae."

Long-Continued and Excessive Trophoblastic Hyperplasia.—Occasionally a peculiar and typical condition develops. After a pregnancy the uterus is found to be enlarged; it is curetted; large masses of trophoblastic tissue and blood clot are removed, and the uterus is apparently emptied. After a week or two, or a month or two, the cycle is repeated, and may be repeated again and again. Lesser degrees of this condition are, of course, relatively fairly common. Proliferating trophoblast that is thus recurrent or present at a time when all trophoblast should have disappeared has been used frequently as a diagnostic index of cancer. We have noted in the literature at least 6 cases in which this process has been pronounced (von Franqué [92l]; Gottschalk [92m]; Meyer,[42] case 1; Handfield-Jones [108]; Vineberg,[109] case 2; Black,[110] case 1). Only 1 of the patients died of frank metastasizing cancer, and in none of them was there perforation of the uterus. Hysterectomy was performed in all, although in 1 case not until after five curettings and after eleven months. Whether the condition would have cleared up ultimately cannot be known, but at any rate the point can be made that the condition is certainly not a constant precursor of frank cancer and that, so far as can be judged from these few examples, proliferating trophoblast behaving in this way is not frequently fatal.

The Frankly Penetrative Trophoblastic Node.—The characteristic lesion of uterine choriocarcinoma is a mass of trophoblastic tissue, with more or less blood clot, which has penetrated some way at least into the myometrium. In some cases it lies buried in the wall, separated from the endometrium by muscle.

We made a table showing the relation between the site and the extent of the lesion found in the uterus and the future behavior of the disease. This table will not be given, partly because it was not possible, from the published descriptions, to assess the uterine lesions satisfactorily. It was quite clear, however, that the table showed nothing of positive

108. Handfield-Jones, M.: Proc. Roy. Soc. Med. **9**:27, 1915-1916.

109. Vineberg, H. N.: Surg., Gynec. & Obst. **28**:123, 1919.

110. Black, W. T.: Am. J. Surg. **20**:638, 1933.

value except the absence of any relation between what one would think to be the degree and the extent of the lesion and the curability of the disease, with the exception, of course, of those lesions which contained villi.

As an example of this, the figures can be quoted for the easily defined group "trophoblastic node completely buried in wall"—a description which must be taken as an index of invasiveness (and, one would think, because of the difficulties of diagnosis, of high lethality). There were 14 such cases in the series. Of the patients, 1 died postoperatively, 3 died with general metastases after hysterectomy and 10 recovered after hysterectomy.

So far as our figures go, given a frank trophoblastic penetration of the myometrium, we find no correlation between the extent or "earliness" of the pathologic involvement found at hysterectomy and the outcome.

THE ASSOCIATED LUTEIN CYSTS OF THE OVARY

A frequent finding in cases of hydatidiform mole and cases of choriocarcinoma is multiple lutein cysts. Such cysts do not occur in every case but are frequent enough to be sometimes of diagnostic help. Presumably, this hyperplasia of lutein cells has a hormonal cause. One can only conjecture what relation is borne by the luteal hyperplasia to the fact that choriocarcinoma occurs in pregnancy, arises from a hormone-producing tissue and is constantly associated with urinary excretion of active gonadotropic substances. A detailed study of the lutein cyst formation associated with choriocarcinoma has been made by Novak and Koff.[111]

METASTASIS OF CHORIOCARCINOMA

The morbid anatomy of choriocarcinoma concerns the distribution of trophoblastic nodes in site and in time. Chorioma means abnormal trophoblastic proliferation; the trophoblastic node is the manifestation of chorioma occurring within the tissues, and choriocarcinoma is that variety of chorioma in which there is or will be dissemination of such nodes.

In this series there were 295 cases of acceptable choriocarcinoma in which metastases were demonstrated. Of the patients, 263 died (complete autopsies were made on 123), and 32 recovered. Cases of nongestational choriocarcinoma are not included in the figures. The organs showing metastases at autopsy were: lung, 158 cases; vagina, 105; brain, 46; liver, 43; kidney, 34; uterine wall, 28; spleen, 24; intestine, 24; broad ligament, 19; ovary, 16; lymph node, 16; "pelvis" (unspecified), 15; cervix uteri, 11; pancreas, 5; skin and subcutaneous tissue, thyroid

111. Novak, E., and Koff, A. K.: Am. J. Obst. & Gynec. **20**:481, 1930.

gland and heart, 4 each; fallopian tube, adrenal gland, bladder, omentum, diaphragm, bone marrow, 3 each; spinal cord, abdominal wall, breast, mediastinum, mesentery, femur and rib, 1 each. In the cases in which the patients recovered, the organs involved by metastases were: vagina, 21 cases; uterine wall, 6; lung (diagnosed roentgenologically), 3; cervix uteri, 2; broad ligament and lymph node, 1 each.

It will be seen that the common sites of metastases are such as to suggest strongly a blood-borne, and not a lymph-borne, spread. Also, curiously enough, there is little direct spread, and we have seen no mention of a choriocarcinoma recurring in the scar after operative removal. This predominantly vascular route could, of course, be explained by referring to what are alleged to be the physiologic properties of trophoblast. No more can be said than that some tumors spread preferentially by the lymphatic channel, some directly and some by the blood stream. Choriocarcinoma happens to belong to the last group. Why it, or any tumor, belongs to this or any other group, no one knows.

As to sites of election, it may be seen that the secondary growths appear by metastasis in the lung, the vagina, the liver, the brain, the kidney and the spleen and by direct or embolic spread in the pelvic tissues. The notable features are the scarcity of metastases in the bones and the lymph nodes, and their relative frequency in the spleen and the vagina. It is usually assumed that spread is, as would be expected, initially to the lungs—perhaps only because clinical and roentgenologic evidence is most obvious there. Certainly mestastases may appear just as early in the vagina as in the lungs.

This tendency for secondary nodules to appear in the vagina is much stronger with choriocarcinoma than with other uterine tumors. It is not stressed even in reports of carcinoma of the cervix uteri occurring during pregnancy—for example, those of Maino, Broders and Mussey [112] and Kobak and associates.[113] It is taken for granted that these vaginal nodules are true metastases in the sense of being embolic and blood borne. Attempts have been made to explain this peculiarly selective behavior by invoking a uterovaginal plexus of veins to be found only in pregnancy. One must be suspicious of such a solitary *post hoc* argument without general validity; furthermore, this anatomic explanation becomes strained when one considers that such nodules may also occur in cases of tubal choriocarcinoma (Dafoe,[114] case 2, for example).

112. Maino, C. R.; Broders, A. C., and Mussey, R. D.: Am. J. Obst. & Gynec. **48**:806, 1944.

113. Kobak, A. J.; Fitzgerald, J. E.; Freda, V. C., and Rudolph, L.: Am. J. Obst. & Gynec. **49**:307, 1945.

114. Dafoe, W. A.: Canad. M. A. J. **40**:376, 1939.

OTHER ASPECTS AND PECULIARITIES OF BEHAVIOR

Besides the general behavior of chorioma, just discussed, there are many peculiarities which separate chorioma from all other neoplasms. Some of these will now be itemized.

Occasional Apparent Absence of a Primary Lesion.—Several cases are recorded in which dissemination of choriocarcinoma occurred (proved either by the appearance of a vaginal nodule or by the findings at autopsy) and yet no primary lesion could be discovered at a gestational site.

In all the cases listed here either no hysterectomy was carried out, or if there was hysterectomy or autopsy, no choriocarcinoma was found in the uterus or the fallopian tube.

1. Vaginal choriocarcinoma which disappeared spontaneously without further trouble:	Pantschenko,[115] case 2.
2. Vaginal choriocarcinoma which disappeared without local excision but after hysterectomy:	Hofmann[93] stated that no such cases were known.
3. Vaginal choriocarcinoma which did not recur after local excision or irradiation; no hysterectomy:	Fleischmann.[92a] Pick.[92b] Schlagenhaufer.[92c] Schmit.[92d] Zagorjanski-Kissel.[92e] Schmitz and Hueper,[116] case 3. Hofmann.[93]
4. Vaginal choriocarcinoma which did not recur after local excision plus hysterectomy:	Mandelstamm,[87] case 2.
5. Vaginal choriocarcinoma progressing to general metastasis and death, with or without hysterectomy:	Hübl.[92g] Lindfors and Vestberg.[92f] Schmorl.[92a] Wehle.[92i] Hicks.[117] Schmauch.[118] Risel.[119]
6. Vaginal choriocarcinoma progressing locally to death:	Meyer,[49] cases 8 and 9.
7. Choriocarcinoma diagnosed elsewhere than in uterus progressing to death with (in nearly all) general metastasis:	Busse.[92n] Heart.
	Kleinhans.[92r] Ovary (? teratoma).
	Marchand.[92w] General spread.
	Schmorl.[92a] Lung.
(This type of case has been studied in detail by Berman[80] and Resnick[79]).	Davis and Harris.[92y] General spread.
	de Zalka.[81] Ovary.
	de Zalka.[81] Liver.
	Novak and Koff.[120] General spread.
	Sears.[121] Jejunum.
	Brews,[122] case 3. Rectovaginal septum.

115. Pantschenko, N. A.: Zentralbl. f. Gynäk. **55**:2744, 1931.
116. Schmitz, H., and Hueper, W.: J. A. M. A. **95**:1413, 1930.
117. Hicks, H. T.: J. Obst. & Gynaec. Brit. Emp. **12**:119, 1907.
118. Schmauch, G.: Am. J. Obst. **50**:88, 1904.
119. Risel: Zentralbl. f. Gynäk. **31**:671, 1907.
120. Novak, E., and Koff, A. K.: Am. J. Obst. & Gynec. **20**:153, 1930.
121. Sears, J. B.: Ann. Surg. **97**:910, 1933.
122. Brews, A.: Proc. Roy. Soc. Med. **28**:1213, 1935.

Brews,[122] case 8. Pouch of Douglas.
Brews,[122] case 15. General spread.
Soresi.[123] Jejunum.
Cron.[124] Broad ligament.
Wilson,[125] case 3. General spread.
Berman.[73] General spread.
Dockerty and Craig.[125a] Brain (no clinical evidence of metastases; no autopsy).
Hamburger,[81a] case 10. Parametrium.
Maun and Green.[126a] Liver (no metastases elsewhere).
Resnick.[79] Breast.

8. Choriocarcinoma in pelvis; death from local spread: Frank.[127]

Such cases evidently occur and have aroused much speculation. In group 7, the "extragenital" cases, it is just possible that some were examples of some tumor other than choriocarcinoma. Cruickshank and Teacher [128] remarked, rightly or wrongly, how mimetic of choriocarcinoma a tumor in the liver might be and described an example. But the accumulated weight of evidence is too great to allow appreciable doubt that such syndromes do occur.

For this peculiarity of behavior we ourselves can offer no explanation. The question could be answered either by assuming regression or disappearance of a cancerous primary focus or by assuming that benign chorionic cells travel to a "secondary" site and there become cancerous. Some support for the first answer is afforded by the findings of Maun and Green [126a]: At autopsy of a patient who had died with multiple metastases of choriocarcinoma these authors found in the uterus only one small area of degenerating trophoblast—the regressing normal placental site—and no neoplasm. They commented [126b]: "If our patient had survived longer, it is probable that the uterus would have been normal, while additional multiple metastatic tumors would have been present at death."

Some support for the second answer is provided by Schmorl's [129] contention that fragments of trophoblast are transported by the blood stream

123. Soresi, A. L.: Am. J. Cancer **28**:583, 1936.
124. Cron, R. S.: Am. J. Obst. & Gynec. **32**:348, 1936.
125. Wilson, K. M.: Am. J. Obst. & Gynec. **38**:824, 1939.
125a. Dockerty, M. B., and Craig, W. M.: Am. J. Obst. & Gynec. **44**:497, 1942.
126. Maun, M. E., and Green, W. M.: (*a*) Am. J. Obst. & Gynec. **46**:738, 1943; (*b*) ibid. p. 743.
127. Frank, R. T.: Am. J. Obst. **74**:369, 1916.
128. Cruickshank, J., and Teacher, J. H.: J. Path. & Bact. **14**:282, 1910.
129. Schmorl, G.: (*a*) Ueber die Puerperaleklampsie, Leipzig, 1893; cited by Veit.[9] (*b*) Verhandl. d. deutsch. path. Gesellsch. **2**:39, 1904-1905; (*c*) Zentralbl. f. Gynäk. **29**:129, 1905.

frequently, if not physiologically, from the placental site to the lungs. This, if true, would still not explain why such cells become cancerous and multiply as cancer cells.

Blood Stream Transportation of Physiologic Trophoblast.—Around the turn of the century there was much interest in the discovery that fragments of placental tissue could become dislodged from the placental site and be carried in the blood stream to the lungs.

Chorionic villi were seen in pulmonary metastases from choriocarcinoma by Solowij and Kryszkowski [92a] and Clemmer and Hansmann [130]; in the lung of a patient with an invasive hydatidiform mole by Miller,[131] and in vaginal nodules by Neumann,[92a'] Pick,[92b] Poten and Vassmer,[92b'] Schmit,[92c] Zagorjanski-Kissel,[92p] Brenner [88] and Meyer [43] (case 8).

Schmorl [120a] found structures closely resembling trophoblast in the lungs of 17 women who had died of eclampsia. Later he [120b, c] stated that he had examined the lungs, and only the lungs, of 158 women who had died at various times of pregnancy and the puerperium. He found embolic displacement of placental cells most frequently in 83 eclamptic women, and in some of them most plentifully, while such displacement was not present or present to much less degree in women dying of other causes. He concluded that a mechanical factor—for example, parturition, uterine contractions or manual removal of the placenta—was not necessary for the dislodgment of the cells, since they could be found even in the first months of pregnancy. He emphasized that he had found evidence of active cell growth in only 3 cases; in 1 case the patient had died of eclampsia ten days after parturition, and in the remaining 2 cases the patients had died after early abortion. He also found cellular proliferation in lung fragments from 2 other women, each with a hydatidiform mole, and in 1 other with a doubtful partial hydatidiform mole.

Kassjanow [132] found emboli of syncytium-like cells in the lungs of 11 pregnant women who had not had eclampsia, and observations similar to those of Schmorl were made by Lubarsch.[133] There was much other work tending to confirm or refute these statements, and the general opinion was summarized in the publications of Veit [9] and Poten.[134]

Veit [9] examined the site of a tubal pregnancy in which the fetus was alive at removal. He found that villi could be followed into maternal

130. Clemmer, J. J., and Hansmann, G. H.: Am. J. Obst. & Gynec. **29**:526, 1935.

131. Miller, J.: Tr. Edinburgh Obst. Soc. **1**:217, 1923-1924.

132. Kassjanow: Dissert. Inaug. St. Petersburg, 1896; cited by Veit.[9]

133. Lubarsch: Fortschritt der Medicin 1893 und zur Lehre von den Geschwülsten und den Infectionskrankheiten; Wiesbaden, 1899; cited by Veit.[9]

134. Poten, W.: Arch. f. Gynäk. **66**:590, 1902.

veins for a distance of 1 cm. Poten [134] considered that the displacing of torn-off villi and their epithelium into the maternal circulation occurred frequently—probably in every pregnancy. He frequently found syncytial fragments and sometimes whole villi in the myometrial veins. He regarded these appearances as the cross section at death of a continuous showering of such fragments from the placental site to the lungs. He concluded, however, that though villal or syncytial displacement is a frequent or constant event, it has neither a physiologic nor a pathologic consequence for the pregnant woman except in chorioma.

These findings have been transmitted down through the textbooks, but there is room for skepticism. Schmorl himself, in his later paper,[120a] recommended caution in interpreting syncytium-like structures in the lung, having seen similar, if not identical, appearances in cases of aspiration pneumonia. Indeed, it is possible that if a parallel series of diseased lungs of men and nonpregnant women had been examined, these appearances might well have been noted. Illustrations of some of Schmorl's material are given by Dunger.[135] Some of them are quite unconvincing; others are certainly suggestive of villi and placental fragments within the lung, but by themselves the illustrations are not sufficient evidence for judgments concerning Schmorl's views. It is necessary, however, to mention here the case reported by Hughes [136] of a woman dying with signs and symptoms of respiratory embarrassment and with a hydatidiform mole still within the uterus. Microscopically, the lungs showed a flooding of the pulmonary circulation with masses of fetal syncytium.

It is worth noting that no work has been done on a scale similar to that of Schmorl's during the past forty years to confirm or refute these tenets. If such trophoblastic embolism of the lung is so constant in eclampsia, it is incredible that it has not become an essential feature of the pathologic aspect of that disease. Also, if there is a constant flooding of villi and trophoblastic fragments into the blood stream in pregnancy, it is remarkable that this has not been reflected in any secondary physiologic or pathologic observations over the past forty years.

Spontaneous Regression of Choriocarcinoma.—Complete spontaneous regression of a proved tumor that is frankly cancer can be taken as being either extremely rare or as not having occurred at all—with the notable exception of cases of choriocarcinoma. Even in cases of choriocarcinoma, and with the reasonable assumption that there is a heavy bias toward the reporting of such cases as apparently do show regression, the incidence is still low. We doubt if there is record of more than

135. Dunger, R.: Beitr. z. path. Anat. u. z. allg. Path. **37**:279, 1904-1905.

136. Hughes, J. E.: Proc. Roy. Soc. Med. **23**:1157, 1930.

20 cases of claimed regression of pulmonary metastases in the whole literature; and, as might be expected, claims of regression of brain metastases are extremely few—we have found only 1, that of Anspach and Hoffman.[137] Even here the authors considered that the symptoms—transitory hemiplegia and aphasia—might have been due to cerebral anemia. There are, of course, many more claims for the regression, or at any rate nonrecurrence, of vaginal metastases.

There is general agreement that it is most difficult to diagnose choriocarcinoma in material from the primary lesion. Because of this difficulty, therefore, it must always be uncertain whether the clearing up, without further treatment, of a syndrome diagnosed on examination of a uterine curetting and on clinical grounds as that of choriocarcinoma was in fact an example of spontaneous regression or whether the diagnosis was mistaken. On the other hand, if the cancerous nature of the tumor has manifested itself in metastasis or in direct spread in the pelvis, the spontaneous disappearance of these secondary tumors or of the primary tumor entitles one to call the case a true example of spontaneous regression.

Regression of Primary Tumor: In some cases, already discussed, there were undoubted metastases of cancer, apparently of a primary gestational choriocarcinoma, and yet no primary lesion could be found at a gestational site. The possible explanations are, first, that there never was a "primary" cancer, the metastases having developed by cancerous transformation *in situ* of noncancerous trophoblast, and, second, that there had been a primary cancer which either had been extruded or had regressed. Since regressions significantly, if rarely, do occur in metastatic growths, it is certainly reasonable to invoke the second explanation.

Regression of Vaginal Metastases: It has sometimes been held—for example, by Veit [9]—that in cases of choriocarcinoma the occurrence of vaginal nodules does not indicate that the lesion is cancerous—mainly, in fact, because these nodules so often regress. This seems to be arguing in a circle. Such vaginal nodules must be "metastatic" by any definition of the term—with, if necessary, the qualification that they differ from most other metastases in that they regress.

That these nodules are trophoblastic in nature, and therefore metastatic, is attested by their macroscopic and microscopic structure, concerning which data are easily obtained at this accessible site. That, without treatment, they sometimes do not grow, and sometimes regress, is well authenticated. Examples of the disappearance of vaginal

137. Anspach, B. M., and Hoffman, J.: Am. J. Obst. & Gynec. 22:239, 1931.

nodules, treated or untreated, in association with absence of a uterine lesion have already been given. Here is a list of examples of recovery despite the occurrence of vaginal metastases in other circumstances.

1. Intrauterine choriocarcinoma; hysterectomy; vaginal metastasis; excision; recovery:	Holzapfel[93c] Karström and Vestberg.[93d] Lonnberg and Mannheimer.[93e] Neumann.[93f] Poten and Vassmer.[93b] Sandberg,[138] 3 months' follow-up. Bremer.[58] Hitschmann and Cristofoletti,[89] case 3. Rosenstein,[139] 4 months' follow-up. Steigelmann,[140] 2 months' follow-up. Mandelstamm,[87] case 4. Laqueur,[45] case 8.
2. Intrauterine choriocarcinoma diagnosed; no hysterectomy; vaginal metastasis; excision; recovery:	Langhans.[93g]
3. Intrauterine choriocarcinoma; hysterectomy; spontaneous disappearance of vaginal metastasis; recovery:	Lackner and Leventhal.[141] Brews,[122] case 52. Hofmann[93] mentions 3 further examples.
4. Tubal choriocarcinoma; salpingectomy; vaginal metastasis; excision or irradiation; recovery:	Klein.[142] Dafoe,[114] case 2.
5. Two cases reported by Pantschenko.[118]	Each patient had a simple hydatidiform mole, and each had a vaginal nodule. One had hysterectomy and excision of the nodule, with recovery; there was only six weeks' follow-up. The other did not have hysterectomy; the nodule disappeared spontaneously and recovery followed. There was, however, no long follow-up.

Regression of Pulmonary Metastases: Our series gathered from the literature includes 12 cases in which regression of pulmonary metastases was claimed to have occurred. In 8 of these the evidence offered for the presence of metastases was clinical only: the appearance and the cessation of hemoptysis, pain in the chest, etc. In 2 cases there was in addition radiologic confirmation. In the remaining 2 cases there was confirmation from biologic pregnancy tests as well as from roentgenographic appearances.

Loopholes could be found in most of these cases when they were regarded separately—some were possibly instances of postoperative

138. Sandberg, K. F. M.: Am. J. Obst. **50**:81, 1904.

139. Rosenstein, W.: Zentralbl. f. Gynäk. **54**:2652, 1930.

140. Steigelmann, L. G.: Zentralbl. f. Gynäk. **60**:1639, 1936.

141. Lackner, J. E., and Leventhal, M. L.: J. A. M. A. **98**:1136, 1932.

142. Klein, P.: Arch. f. Gynäk. **129**:662, 1926.

pulmonary infarction—but their aggregate evidence confirms the belief that pulmonary metastases can regress. The relevant reports are:

Clinical evidence only:	Chrobak.[92b]
	von Franqué.[921]
	Kworostansky.[92j]
	Ladinski.[92j]
	Lonnberg and Mannheimer.[92o]
	Pestalozza.[92k]
	Zagorjanski-Kissel.[92p]
	Phillips.[143]
Clinical plus radiologic evidence:	Klein.[142]
	Lackner and Leventhal.[141]
Clinical plus radiologic plus hormonal evidence:	Mazer and Edeiken,[144] case 1.
	Peightal.[145] This patient died of cerebral hemorrhage due to metastasis.

It is appropriate to mention here the account given by Maier and Taylor [146] of a patient with what was stated to be a solitary metastasis of choriocarcinoma occurring in the lung two and a half years after passage of a hydatidiform mole. This secondary tumor was removed by lobectomy, with evident cure.

Regression of Metastases or of Direct Extensions at Other Sites: The remaining groups are self explanatory:

1. Intrauterine choriocarcinoma; hysterectomy; metastasis other than vaginal; removal; recovery:	Cazin-Segond.[92j] Ovary.
	Kolomenkin.[92m] Beside cervix uteri.
	Laqueur,[45] case 8. Mesovarium.
2. As in group 1, but intrauterine choriocarcinoma was presumptive since hysterectomy was not done (lesion treated by irradiation):	Schmitz and Hueper.[116] Kidney.
3. Intrauterine choriocarcinoma; hysterectomy; metastasis in brain diagnosed clinically; recovery:	Anspach and Hoffman.[137]
4. Tubal choriocarcinoma; salpingectomy; metastasis other than vaginal not removed; recovery:	Phillips.[143] Mesenteric lymph nodes.
5. Intrauterine choriocarcinoma; hysterectomy; apparently, cancer was incompletely removed; recovery:	Marchand.[60a] Parametrial veins.
	Noble.[147] Beside bladder.
	Hitschmann and Cristofoletti,[89] case 3. In pelvis.
	Davis and Brunschwig.[97] Cervical stump.

143. Phillips, M. H.: J. Obst. & Gynaec. Brit. Emp. **20**:299, 1911.
144. Mazer, C., and Edeiken, L.: Am. J. Obst. & Gynec. **26**:195, 1933.
145. Peightal, T. C.: Am. J. Obst. & Gynec. **28**:435, 1934.
146. Maier, H. C., and Taylor, H. C.: Am. J. Obst. & Gynec. **53**:674, 1947.
147. Noble, C. P.: Am. J. Obst. **53**:846, 1906.

There can be no doubt that sometimes choriocarcinoma, either the primary tumor or its metastases, or both, can undergo spontaneous regression. Since it occurs, the regression is presumably reflected in some histologic changes. Littauer [148] mentioned Risel as having found, in a patient who had died with pulmonary metastases, areas in the lung where the cancer tissue was being "choked" by granulation tissue. Teacher [149] also gave a detailed description of the same process.

"Spontaneous regression" may be expressed differently by saying that the rate of growth is irregular and changeable as compared with that of other tumors. The whole structure of cancer therapy is, of course, based on the assumption that any tumor will continue to grow at the same rate after diagnosis as before.

CHORIOCARCINOMA OF THE FALLOPIAN TUBE

According to the figures in table 1, the mortality rate for all conditions diagnosed at any time as choriocarcinoma of the fallopian tube is higher than the rate for choriocarcinoma of the uterus. The mortality rate is, however, lower than that for all conditions diagnosed at any time as choriocarcinoma following full time pregnancy. This suggests that the abnormal situation of the ovum per se is not a factor contributing to the relatively high mortality rate. It is also possible that the increased difficulty of diagnosis of choriocarcinoma of the tube is compensated by the decreased opportunity for overdiagnosis.

Otherwise there is little to add. Choriocarcinoma of the fallopian tube behaves much as one would expect choriocarcinoma to behave when in the tube.

Here is a list of authors who have reported cases:

Ahlfeld.[92a]
Albert.[92o]
Gebhardt.[92p]
Nickiforoff.[92q]
Rosner.[92r]
Senarclens.[92s]
Phillips.[148]
Cope and Kettle.[150]
Klein.[143]
Chatunzew.[151]
Georgii.[152]
Stein.[153]
Grudzew and Russky.[154]
Thomas.[155]
Bunnag and Bachman.[156]
Williams.[157]
Tractenberg.[158]
Pearse and Fraser.[159]
Fleming, Thompson and Fox.[160]
Dafoe,[114] 2 cases.

148. Littauer, A.: Arch. f. Gynäk. **72**:294, 1904.
149. Teacher, J. H.: J. Path. & Bact. **12**:487, 1908.
150. Cope, V. Z., and Kettle, E. H.: Proc. Roy. Soc. Med. **6**:247, 1912-1913.
151. Chatunzew, W. A.: Monatschr. f. Geburtsh. u. Gynäk. **86**:60, 1930.
152. Georgii, S.: Zentralbl. f. Gynäk. **54**:2003, 1930.
153. Stein, H. E.: Am. J. Obst. & Gynec. **23**:416, 1932.

(Footnotes continued on next page)

Williams [157] reported that up to 1938 the total of recorded examples was 41.

CAUSES OF DEATH IN PATIENTS WITH CHORIOCARCINOMA

Sometimes death occurs while the tumor is still localized. Fatal intraperitoneal hemorrhage has been reported on a few occasions. The article by Anspach and Hoffman [107] deals particularly with this type of case; one of the most recent examples is that described by Rosenbloom.[107]

Apart from cases of death from fatal intraperitoneal hemorrhage and the much commoner cases of death from intrauterine sepsis or external hemorrhage it is remarkable how rarely one reads of death due only to local pelvic extension of the tumor. The case described by Adachi [161] was of this type. Hicks [117] and Caturani [73a] reported similar cases, though even in those there was evidence of terminal pulmonary metastasis.

Death usually occurs with a distribution of trophoblastic nodes involving several organs and is presumably due to resultant interference with function. It is interesting to note here a curious paradox. The patient who dies does so, on an average, two to three months after a hysterectomy which showed the lesion apparently still localized to the uterus, and it appears that fatal choriocarcinoma is the most rapidly killing tumor of all. Yet there is the fact that the cells of choriocarcinoma resemble their physiologic prototype more closely than do those of almost any other malignant tumor. The criteria of cancer are absent.

We have suggested elsewhere that this paradox may be due to the fact that a large amount of hemorrhage is usually associated with relatively few trophoblastic cells, but it is doubtful if even this explains the rapid lethality. It does look as though trophoblastic cells produce some peculiar "toxins," presumably hormonal, or, alternatively, that the trophoblastic proliferation itself is a manifestation of some acute systemic dysfunction, again presumably hormonal.

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REPORT ON TWENTY-THREE PATIENTS WITH CHORIOCARCINOMA

The data and material for this report were found in the files of the Royal College of Physicians' Laboratory, Edinburgh, and of the department of obstetrics and gynecology of the University of Edinburgh.

Altogether there were records of 45 patients who, over the past sixteen years, had been judged, certainly or tentatively, to have choriocarcinoma. On 37 of these, adequate follow-up data were obtained. In 23 of these 37 the clinical findings had sufficiently conformed with the histologic diagnosis to justify the patients' undergoing hysterectomy for choriocarcinoma. Theirs are the cases now reported as examples of choriocarcinoma. Six of these patients died of the disease, giving a mortality rate of 25 per cent.

Nine of the remaining 14 patients did not have hysterectomy performed and are alive and well. The remaining 5 died of lesions unconnected with choriocarcinoma except so far as these lesions raised for a time a false suspicion of choriocarcinoma.

The main features of these cases are shown in table 6.

The most striking fact about these cases is that, with the possible exception of cases 14 and 15, they are all straight-forward and typical. As remarked earlier, it was impossible to discover from the literature just how relatively frequent were those cases the peculiarities of which have given the disease its notoriety and interest. These 23 cases which we report confirm the impression that in the great majority of instances choriocarcinoma behaves in an "ordinary" way and that peculiarities, such as regression, are relatively rare.

Our series also confirms the frequency with which choriocarcinoma is diagnosed nowadays after hydatidiform mole has developed, and the relative noncancerous nature of such conditions. It also stresses the high prognostic value—for absence of canceration—of the presence of chorionic villi in the lesion, strikingly seen in case 14, in which an endocervical mass was treated with radium and a vaginal node excised, and yet the patient recovered.

ETIOLOGIC FACTORS

As far as choriocarcinoma situated in the uterus, fallopian tube or vagina is concerned, no one has suggested that it ever arises other than after pregnancy, and there is no evidence that it ever does. There is this blunt fact. There is the proportional frequency of choriocarcinoma following different types of pregnancy, and there are the data established in regard to hydatidiform mole. Apart from these nothing is known of the factors which determine whether a pregnancy will result in a choriocarcinoma. In fact, there is so little that there is almost nothing to discuss. Such factors as have been suggested have been chosen because of

TABLE 6.—*Summary of the Findings in 23 Cases of Choriocarcinoma*

Case	Age of Patient	Parity	Associated Pregnancy	Interval: Cessation of Pregnancy to Hysterectomy	Lesion Found at Hysterectomy	Follow-Up	Presence of Villi in the Removed Uterus or in the Diagnostic Curettings
1	34	4	Hydatidiform mole	2 mo.	Penetrating hydatidiform mole	Alive and well 14 yr. later	+
2	23	1	Hydatidiform mole	4 weeks	Penetrating trophoblast	Alive and well 9 yr. later	0
3	27	1	Hydatidiform mole	10½ mo.	Large trophoblastic node, 2.5 cm. in diameter, deep in myometrium	Alive and well 8 yr. later	0
4	50	8	Hydatidiform mole	?	Penetrating hydatidiform mole	Alive and well 9 yr. later	+
5	28	7	Hydatidiform mole	2 mo.	Trophoblastic node, 2 cm. in diameter, in myometrium beneath endometrium	Alive and well for 4 yr., then died of lobar pneumonia	0
6	30	4	Hydatidiform mole	2 mo.	Penetrating trophoblast	Alive and well 3 yr. later	+
7	43	4	Hydatidiform mole	2 mo.	Penetrating hydatidiform mole	Alive and well 6 yr. later	+
8	27	2	Abortion	c.6 mo.	Trophoblastic node, 2 cm. in diameter, in myometrium	Died 1 mo. after hysterectomy; no autopsy but death probably due to metastases	0
9	29	0 or 1	Hydatidiform mole or abortion	1⅛ yr. or 5 mo.	Trophoblastic node, 1.5 cm. in diameter, in myometrium + vaginal node, 3 cm. in diameter	Died 4¼ mo. after hysterectomy; autopsy showed metastases in lungs and brain	0
10	21	0	Hydatidiform mole	4½ mo.	Penetrating (?) trophoblast	Alive and well 2 yr. later	+
11	26	2	Full time pregnancy	6 mo.	Massive trophoblastic invasion of myometrium + vaginal node, 2 cm. in diameter	Died 5 mo. after hysterectomy — clinically, with lung metastases	0
12	21	1	Hydatidiform mole	1½ mo.	Two trophoblastic nodes in myometrium, each about 2 cm. in diameter	Alive and well 5 yr. later	+
13	31	2	Hydatidiform mole	2 mo.	Trophoblastic node, 3 cm. in diameter, in myometrium	Alive and well 15 yr. later	+
14	26	2	Abortion	2 mo.		Alive 4 yr. later	+
15	55	2+	Abortion	3 yr. from last period and abortion 1 yr. before that	Trophoblastic node, 2 cm. in diameter, in myometrium	Advanced mammary carcinoma removed 1½ yr. after hysterectomy, death 2 yr. later with roentgenologic evidences of metastases in brain and spinal cord	0
16	30	1	Hydatidiform mole	3½ mo.	Trophoblastic node, 3 cm. in diameter, deep in myometrium + a parametrial node	Alive and well 10 yr. later	0
17	45	10	Hydatidiform mole	0	Trophoblastic erosion	Alive and well 8 yr. later	+
18	26	3	Full time pregnancy	1 mo.	Trophoblastic node, 1 cm. in diameter in myometrium	Alive and well 7 yr. later	0
19	32	7	?	?	Trophoblastic node, 1 cm. in diameter, in myometrium; more trophoblast near cervix	Alive and well 6 yr. later	+
20	31	3	Abortion	2 yr.	Very large trophoblastic node occupying fundus + smaller trophoblastic node in parametrium	Died 10 mo. later with clinical evidence suggesting lung metastases	0
21	26	4	Full time pregnancy	10 days	No choriocarcinoma found	Alive and well 14 yr. later	0
22	25	0	Full time pregnancy	5 mo.	Reported with a pelvic mass and a vaginal nodule. Regarded as inoperable. Roentgen therapy	Died 2 mo. after start of roentgen therapy; autopsy showed large tumor of uterus and vagina, metastases in lung and liver—all typical of choriocarcinoma	0
23	42	4	Probably hydatidiform mole	6 mo.	Several trophoblastic nodes, 1 to 9 cm. in diameter, in myometrium	Died 3 mo. later with metastases in vagina and (clinically) lung	0

the possibility that in effect they reduce maternal resistance to the "invasion" of trophoblast—for example, syphilis, anemia and "endometritis"—but none of these has ever been found to be truly relevant. Such agencies as "defensive" syncytiolysins have never been isolated.

The conception which forms the background for current opinion respecting choriocarcinoma can be strongly criticized. Most authorities have, in some cases quite explicitly, formed their theories of choriocarcinoma on the following syllogism: Trophoblast is an active, invasive and physiologically malignant tissue; therefore choriocarcinoma is a highly invasive and malignant tumor. Implied is the major premise that highly active, "invasive" physiologic tissues form highly active cancers. Both propositions may be denied.

The first, that trophoblast is invasive in any crude physical way, has already been fully discussed and the opinion expressed that it is not so. The second proposition is certainly wrong. The facts concerning the behavior of individual tumors are the most casual and accidental aggregation of unrelated facts in the whole of medicine. In the present state of knowledge we can perhaps differentiate physiologic tissues into more or less rapidly growing or more or less blatantly functioning classes, and to some extent the tumors derived from these tissues. Melanocarcinoma, which in its irascibility and frequent fulmination approximates in behavior choriocarcinoma, has as its prototype nevus cells which from the point of view of function and proliferation appear to be practically inert. Some tissues, like trophoblast, postmenstrual endometrium, lymphoid germinal centers and granulation tissue, are rapidly proliferating. Some tumors occur frequently and some rarely. Some tumors as a type are highly cancerous, and some are benign. And choriocarcinoma occupies its place in this chaotic distribution as a tumor which occurs very rarely, may be highly cancerous and has a rapidly growing prototype. That is all that can be said. Indeed, most of the facts of choriocarcinoma are just empiric facts, no more explainable than most of the facts in the pathology of cancers as a whole.

NATURE OF THE DISEASE

The most important ways in which the behavior of chorioma differs from that of neoplasms in general are:

1. It occurs only during or after pregnancy.

2. It is constantly associated with hormonal changes, so much so, in fact, that these changes can be taken as an index of the presence of trophoblast. Other endocrine tissues may form "functioning" cancers, but in none of these is the functioning element so constant or so prominent as in chorioma.

3. There is no apparent difference as to the cell type between chorioma which shows little invasion and chorioma which is frankly choriocarcinoma.

4. True regressions occur in a small but certainly significant proportion of cases.

5. Chorioma is regarded, rightly or wrongly, as the only tumor occurring in the body whose cells have a different genetic constitution from those of the host.

6. Choriocarcinoma kills either within twelve months of diagnosis or not at all.

RELATION TO PREGNANCY

The question is: Is chorioma merely the cancer of trophoblast and therefore associated with pregnancy only to that extent, or might it be that dysfunction of the endocrine readjustment of pregnancy plays a small, large or dominant part in causing chorioma to develop or in causing it to extend into choriocarcinoma?

There would be two ways of trying to assess how far pregnancy itself affects the behavior of the chorionic epithelium:

1. By assuming that the systemic changes of pregnancy are different, perhaps more, perhaps less, in, say, a two month abortion, a three month hydatidiform mole, a full time pregnancy and six months after parturition and trying to discover whether there are any correlated differences in incidence and behavior of choriocarcinoma developing at these times.

2. By trying to discover whether other tumors show differences of behavior if they occur during pregnancy.

As to the first method it has already been pointed out that the risk of choriocarcinoma developing from trophoblast is apparently equal throughout the life of the trophoblast. There is no evidence that the time of development makes any difference in the behavior of choriocarcinoma. It is said that choriocarcinoma occurring after hydatidiform mole is less malignant in its behavior than after other types of pregnancy, but this is doubtful.

As to the second method, it is well known that in some tumors—for example, carcinoma of the breast—pregnancy accelerates growth and increases malignancy. Mostly, however, it can be said that pregnancy has little effect on tumor growth.

No more can be said, therefore, than that choriocarcinoma occurs during pregnancy and that its incidence and behavior are not related specially to the type or the "degree" of pregnancy.

THE ENDOCRINE BACKGROUND

In most cancers there is a microscopic picture of polymorphism of cells, hyperchromatism, mitotic figures, etc., which gives the impression

that the property of cancer lies in the cells themselves. In chorioma, however, we find a tissue, trophoblast, which looks the same as physiologic trophoblast and apparently is the same in the wide continuum of trophoblastic behavior stretching from the benign, self-extruding hydatidiform mole to fulminating choriocarcinoma. This and the occasional but significant regression and the constancy of hormone production combine to give an impression that the behavior of chorioma is conditioned by a balance of forces in a way that the behavior of other tumors is not.

Further, the fact that in general the course of the disease is limited to six months or so also suggests an acute dysfunction initiated only during the state of pregnancy and lapsing as the changes of pregnancy disappear. The constancy with which the result of the biologic pregnancy test is positive and the frequency with which luteal cysts are associated with proliferating trophoblast suggest strongly that this hormone production, even if the hormone is only a by-product, is a manifestation of the mechanism which results in the proliferation of trophoblast. This hormone occurs physiologically and, as such, occurs as part of the endocrine upheaval of pregnancy. Many metaphors have been used to describe the endocrine system—"team," "orchestra" and the like. Common to these is the idea that each hormone acts on, and is acted on by, every other hormone. In chorioma the proliferation of trophoblast is constantly associated with the production of a hormone which is an integral part of the endocrine setup of pregnancy. In the conventional concept of chorioma this production of gonadotropin is a necessary but, apart from purposes of diagnosis, unimportant by-product and the essential nature of choriocarcinoma is that of a neoplasm which grows, metastasizes and kills as do other cancerous neoplasms. As far as present knowledge goes, this is, of course, substantially correct. One might well suspect, however, that all this production of hormone would have endocrine causes and effects and interrelationships; indeed, the peculiarities of choriocarcinoma, quite apart from its possible fetal nature, which separate it off from all other tumors, also point vaguely in this direction—namely, that there is an endocrine background to the causation and behavior of the trophoblastic proliferations which is still to be worked out. Although there are plenty of hints that this is so, there is as yet little real evidence.

RELATIONSHIP OF CHORIOMA AND OTHER NEOPLASMS

In these peculiarities which we have just described chorioma obviously runs parallel to certain other tumors arising in endocrine organs, which we may call the "endocrinomas."

The properties of tumors of this class are: 1. They arise in endocrine organs. 2. They are formed of mature cells which individually are simi-

lar to the prototype cells and together form a structure more or less similar to that of the prototype tissue. 3. They produce the appropriate hormone, which has pathologic effects similar to those produced physiologically. 4. At their lower levels of malignancy they graduate into simple hyperplasias with hyperfunction. 5. When, as sometimes happens, the new growth behaves like a cancer, showing invasiveness and even metastasizing power, the tumor cells retain their physiologic-morphologic characteristics and structure. As a result, it is not possible to foretell cancerous behavior from histologic aspects alone. 6. Sometimes they occur as ectopic growths in the gonads. Such occurrence may be associated with a relative frequency of appearance in cases of teratoma.

This concept of "endocrinomas" could be greatly expanded. Meantime to this class we would allocate: acidophil and basophil adenoma of the pituitary gland; some examples of thyroid adenoma, including "benign" metastasizing thyroid adenoma (toxic goiter may have a place here); thymoma of the type which maintains thymus structure; adrenal cortical adenoma; ovarian granulosa cell tumor; some examples of arrhenoblastoma; possibly Leydig cell tumor of the testis; chorioma.

Tissues resembling these endocrine hyperplasias have a predilection for gonadal or teratomatous sites—struma ovarii, choriocarcinoma testis and ovarii, and corticoid arrhenoblastoma of the ovary. It may be that other such tissues occur there but are not recognized.

In all these tumors there are many hints that the hyperplasia or neoplasm is a manifestation, possibly intermediate, of endocrine imbalance. The tumor most closely analogous to chorioma and choriocarcinoma is the granulosa cell tumor of the ovary.

Chorioma would in this sense be the simple hyperplasia, and choriocarcinoma the cancerous hyperplasia, of the endocrine organ formed in the uterus in pregnancy.

HISTOGENESIS OF CHORIOCARCINOMA

Choriocarcinoma was considered to be a tumor of maternal origin until the publication of Marchand's papers, in 1895 and 1898. Since then its fetal origin has been completely accepted. There is still, however, sufficient evidence of a maternal influence on its genesis and growth to make a reexamination of the evidence for and against worth while. There are two preliminary considerations.

1. It is not a logical argument, but it can be said that if choriocarcinoma is to be unique among all the types of tumor of the body in that it arises from fetal cells, the onus of proof lies in showing that it is in this way different from every other tumor. This applies to every phenomenon that is unique in lying just outside a large class.

2. The fundamental fact is that the structure of choriocarcinoma resembles that of trophoblast, and trophoblast is universally accepted

to be wholly fetal in origin. By conventional use of the term, choriocarcinoma is "derived from" trophoblast. The histogenetic classification of tumors is, in fact, no more than a convenient notation for describing what is seen under the microscope. All that its terms say is that there are neoplastic tissues which resemble prototype tissues more or less closely. It says nothing of the mechanism which has brought about this similarity even at the same site. Are there precursor cells with an internal constitution so determined that if they divide physiologically they produce a certain structure, while if they divide neoplastically they produce tissue of a more or less similar structure? Or are there precursor cells which are constitutionally indifferent but which are caused to differentiate to a common underlying pattern, either physiologic or cancerous, by local environmental influences which may vaguely be called tissue inducers or determinants? Such distinctions are unimportant in "deriving" a maternal tumor from a maternal prototype tissue, but they are fundamental in the genesis of choriocarcinoma.

Assuming that form is a reflection of function, and assuming that tissue with the morphologic features of trophoblast is determined at the placental interface by impulses arising out of local functional needs, and assuming that many of the cells so acted on are fetal in origin, there seems to be no reason a priori why similarly situated maternal cells should not also react to the same functional impulses and in the same way to produce morphologic trophoblast.

BEHAVIOR OF OTHER FETAL TUMORS

The only tumor which is claimed to be of fetal origin and to invade the maternal host is choriocarcinoma. There are no other such fetal tumors—a fact in itself of significance.

HOMOLOGOUS SPREAD OF HUMAN TUMORS

Homologous transplantation of tumor tissue is easy in certain animals —for example, mice. In other, even closely related species both carcinogenesis and tumor transplantation may be difficult and rare. As far as is known, homologous transplantation practically never occurs in man. Many perverted attempts have been made to transplant such diseases as leukemia, and, of course, many opportunities have occurred accidentally and naturally for tumor fragments to be dislodged from one individual and inoculated into another. Willis [162] has said: "Our experience of the transfer of animal tumors from one individual to another by inoculation should engender caution in denying the possibility of this event occurring accidentally in human beings. However, no conclusive example of such transfer has so far been reported."

162. Willis, R. A.: The Spread of Tumours in the Human Body, London, J. & A. Churchill, Ltd., 1934, p. 97.

We have seen no report of a fetal tumor frankly invading the maternal tissues. It is well known that a mother bearing a child with congenital leukemia is herself unaffected by the disease, and also that the converse holds—a leukemic mother always bears a nonleukemic child. Wells [163a] reviewed the relevant literature and could find only 4 acceptable cases in which new growth was found in the fetus or child secondary to carcinomatosis (lymphosarcoma in 1 of the 4 cases) of the mother. The reverse process, from fetus to mother, must be extremely rare if indeed it has ever occurred. Wells made no mention of such a happening in his review.

RELATIVE FREQUENCY OF CHORIOCARCINOMA

We have to decide whether chorioma is a neoplasm arising from tissue 2 to 12 months old or a neoplasm arising from tissue 20 to 50 years old, dating from the fertilization of the ovum.

Congenital tumors occur which are manifest either at birth or later with the strong presumption that they started before birth. These tumors are mainly sarcomas or neurogenic retinoblastomas or neuroblastomas. Regarding carcinomas, Wells [163b] examined the evidence and concluded: "Lacking more positive evidence, one may be justified in doubting that congenital carcinoma ever has been observed, and certainly none of the types common in adult life."

On the other hand, carcinoma and other tumors occurring in women during pregnancy between the ages of 20 and 50 are not uncommon; certainly, they are far commoner than congenital fetal tumors. It can therefore be said, all other things being equal, that if there is no evidence indicating whether the cell type of origin is fetal or maternal, the probability is, again other things being equal, that the cell type is an adult one.

This argument is enormously strengthened by the great rise in incidence of choriocarcinoma per thousand cases of maternity which starts at the age of 40. This rise is almost exactly parallel to that which occurs in the age incidence of many carcinomas which are rare in the 20's, less rare in the early 30's, and which at any age between 35 and 40 increase rapidly in frequency to reach a peak incidence between 45 and 55. It may be that one would have to distinguish two factors, a tumor tissue of fetal origin and a maternal tissue which offered less resistance to invasive spread at higher ages.

NATURE OF TERATOGENOUS CHORIOCARCINOMA

In teratomas there occurs, along with tissues like glia and cartilage, which are accepted without debate as hostal in origin, tissue which, whether it is called trophoblast or not, undoubtedly resembles tropho-

163. Wells, H. G.: (*a*) Arch. Path. **30**:535, 1940; (*b*) ibid. p. 583.

blast. It does seem that hostal tissue can produce tissue morphologically and hormonally identical with trophoblast just as it produces tissues that morphologically are glia and cartilage.

SOURCE OF CHORIONIC GONADOTROPIN

The production of chorionic gonadotropin is a characteristic function of trophoblastic cells and is a diagnostic index of the presence of chorio-matous trophoblast as well as of pregnancy. As has been discussed earlier, it has not been found possible to distinguish the endocrine substances produced in association with (*a*) pregnancy trophoblast, (*b*) gestational choriocarcinomatous trophoblast, (*c*) teratomatous "trophoblast" and (*d*) occasional testicular tumors other than choriocarcinoma. It appears, then, that what one would imagine to be the most highly specialized function of trophoblast, the production of chorionic gonadotropin, can be carried out by cells of hostal as well as by cells of fetal origin. The possibility is at least admissible that the parallel specific morphologic characters may also be formed by hostal as well as by fetal cells. There is evidence that in the pregnant mare gonadotropin is produced by maternal and not fetal cells.

HISTOLOGIC ASPECTS OF CHORIOMA

Trophoblast has two constituents. So far as the Langhans' cell masses are concerned, these tend to merge into, or be indistinguishable from, tissue which is undoubtedly maternal, so that it would be difficult to establish the morphologic identity of Langhans' cells alone. Langhans' cells can be, and usually are, identified, however, by the syncytium which is constantly associated with them. There has been much debate regarding the interrelationship of these two constituents, but the tendency now is to regard the Langhans cells as the active proliferative element and the syncytium as in some way one of its functional modes. Meyer [43] held that syncytium appeared whenever the trophoblast had adjacent fluid nutrition and that syncytium was always and only found lining clefts and cavities.

We can refer here to our case 11, in which the first curettings showed masses of tissue which was cancerous by all the histologic standards by which one diagnoses cancer elsewhere. There was no syncytium, and the original histologic diagnosis could only be that of an aggressive cancer. It certainly did not show morphologic characters diagnostic of a trophoblastic origin, and yet in every other respect the history and the subsequent course were typical of choriocarcinoma, including the appearance of a vaginal node containing trophoblast of both types.

If cytotrophoblast and cells of undoubtedly maternal origin can merge and be confused with one another, as in chorioma and in the early stages of placentation particularly, and if the distinctive feature of trophoblast

is the syncytial mode which expresses some function, the question again immediately arises: Is the localization of this functional structure conditioned by the functional needs peculiar to the neighborhood of the villus, or is it conditioned by the inherent genetic constitution of the cells?

PECULIARITY OF BEHAVIOR

Another part of the background for the current theory of origin is the peculiarity of behavior of choriocarcinoma. This would presuppose, or at least support, a peculiarity of origin. Much of the peculiarity of choriocarcinoma is no more than the individuality of behavior which every tumor shows, but there is a residuum of behavior—for example, its regressibility—which separates choriocarcinoma from all other tumors as a class. To this extent it is unique. But there is no need to invoke a derivation from fetal cells as the only associated uniqueness. Choriocarcinoma is also unique in that, whether of fetal or of maternal origin, it arises only during or after the pregnant state.

ANALOGY WITH OTHER ENDOCRINE NEOPLASMS

We have described how closely in its behavior choriocarcinoma, or rather chorioma, resembles other tumors arising in endocrine organs, particularly granulosa cell tumor. Indeed, the resemblance to granulosa cell tumor includes latency of development. Just as granulosa cell tumor frequently appears after the menopause, so chorioma frequently appears after the end of pregnancy. The similarity in behavior of these two neoplasms suggests that they are subject to similar neoplastic influences. These influences might, of course, act equally well on fetal and on maternal cells, and since the influences act peculiarly on endocrine cells—and fetal trophoblastic cells may be regarded as part of an endocrine organ peculiar to the pregnant uterus—this could be used as evidence for a fetal origin of chorioma; but the influences themselves cannot be fetal in the case of granulosa cell tumor at least.

ANALOGY WITH BEHAVIOR OF PHYSIOLOGIC TROPHOBLAST

It is possible that the now unargued acceptance of the origin of choriocarcinoma from fetal cells is due largely to the neatness of the conventional theory: There is trophoblast which is invasive; therefore, it must be foreign and fetal; sometimes the defensive barriers break down, the trophoblast continues to invade, and that is choriocarcinoma. As has been discussed, trophoblast is certainly not invasive in any crude physical way, and there is in neoplasms in general no correlation between degree of canceration and behavior of the physiologic prototype tissue.

Whether this cumulative evidence indicates a maternal origin for choriocarcinoma, and if so how this is to be reconciled with the fetal

origin of physiologic trophoblast, we are not prepared to say. Certainly the problem is not so simple as not to be worth discussion.

Since all the somatic cells of a human being have the same sex, if it were technically possible to demonstrate that all trophoblastic cells were always female, or sometimes male and sometimes female, this would be absolutely conclusive proof one way or the other.

DIAGNOSIS

The difficulty of diagnosis is that the trophoblast found in simple hydatidiform mole is similar to that in fulminating choriocarcinoma.

Suspicion of choriocarcinoma may be aroused by the symptoms themselves or by a preceding history of hydatidiform mole. A history of hydatidiform mole has no other function and should be allowed no weight at all in the question whether a trophoblastic hyperplasia is, or will become, cancerous.

The presence of trophoblastic hyperplasia is established by (*a*) microscopic examination and (*b*) biologic pregnancy tests.

Occasionally there is a pointer to the potential of the trophoblast—many mitotic figures, a situation in sheets deep within the myometrium and the presence of chorionic villi. It is the main duty of the histologist, and no more so far as he is a histologist, to inform the clinician of these and any other findings. Sometimes, too, the behavior of the biologic pregnancy test titer will help.

In view of all the data available to him the clinician must then assess the probabilities and answer the following questions:

1. Is trophoblastic hyperplasia present?

2. How fast is the trophoblast proliferating and how far has it extended? Apart from the vexed question of vaginal nodules, the presence of metastases is, of course, an immediate answer. Other than this the position is usually difficult.

3. Having decided as best he can that there is still present trophoblast which is continuing to proliferate, and having located it in either the uterine cavity or the uterine wall, the surgeon must then ask himself: How will this tissue behave if left untreated? For this he has little more to work on than the assumption that the trophoblastic proliferation will continue at the same rate and that an actively growing and locally invasive trophoblast will progress and disseminate. In other words, his evidence is that trophoblast which is found abnormal in site has invaded to that site; and that trophoblast which is found abnormal in time is trophoblast which has continued to proliferate abnormally for that time. He can only assume that these processes will continue at the same rate.

TREATMENT AND CURABILITY

These two subjects are intimately connected in cases of chorioma. If treatment has been decided on, there is general agreement that it should be surgical. Postoperative radiotherapy may be used as an adjunct. Various other forms of treatment have been tried, usually in the occasional case in which a woman has refused hysterectomy, based on Fraenkel's [164] syncytiolysin theory. They include such procedures as transfusion of the blood of a healthy pregnant woman (Richter [165]) and the giving of placental tissue extracts (Massaza [166]).

Manifest choriocarcinoma is a highly malignant tumor, but it is reasonable to hold that many patients who would otherwise have died are cured by treatment. On the other hand, it is often difficult to know that this is so, even after hysterectomy and follow-up, so that the surmise is unavoidable that many uteri must have been removed from women who would not have died if left untreated.

Hitschmann and Cristofoletti [59] emphasized the dangers of operative manipulation as such in causing premature dislodgment of cancerous emboli. They attempted to prove this danger by comparing the incidence of metastases (additional to those in lung and vagina) in women who died of choriocarcinoma at varying times without operation and in women who died of choriocarcinoma four to five weeks after operation. Since they were comparing all the patients who were not operated on with the 18 (of 300) most rapidly dying patients, presumably afflicted with the most malignant neoplasms, who were operated on, their deductions were not valid. In any case it is now generally agreed that the dangers of biopsy in disseminating the cancer have been greatly exaggerated.

Respecting choriomas, there is no exactness in definition, diagnosis or prognosis, or even in the assay of the beneficial effects of treatment, such as obtains with other neoplasms. There is, therefore, a place for ordered temporizing and delay such as does not obtain with other neoplasms, and there is no doubt that the tendency nowadays is toward increased conservatism.

Endocrine Therapy.—The trophoblastic hyperplasia and the chorioma seem to have an intimate relation to endocrine dysfunction. Indeed, it is not known which of their manifestations are cause and which effect. We have called chorioma "endocrinoma" and have considered it as the

164. Fraenkel, E.: Neue folia Gynäk. **180**:69, 1897; cited by Hertig and Sheldon.[94]

165. Richter, W. E.: Zentralbl. f. Gynäk., 1939, abstracted in J. Obst. Gynaec. Brit. Emp. **46**:1065, 1939.

166. Massaza, M.: Atti Soc. ital. di ostet. e ginec. **33**:91, 1937. (not available and noted by title only); Ginecologia **3**:399, 1937 (not available and noted by title only).

hyperplasia of an endocrine organ peculiar to pregnancy. It produces a demonstrable excess of the hormone of pregnancy just as granulosa cell tumor produces a demonstrable excess of estrogen. Although their actions are complementary, estrogen and chorionic gonadotropin ("pregnancy hormone") have opposite effects physiologically. It would be reasonable to try the effect of large doses of synthetic estrogens on patients with chorioma.

SUMMARY

A series of 516 reported cases of choriocarcinoma, including 23 reported in this article, are analyzed. The following points are discussed:

1. Definition. An abnormal proliferation of the trophoblast has been classified as chorioma, and the cancerous variety as choriocarcinoma.

2. Incidence relative to age and to parity. It was found that the frequency of choriocarcinoma per thousand maternities increased greatly with age but that parity had little or no relation to the incidence.

3. The relative frequency with which choriocarcinoma is preceded by different types of pregnancy.

4. The curability of choriocarcinoma. The curability rate has risen considerably in recent years. This is almost entirely due to the increased curability of choriocarcinoma following hydatidiform mole. The possibility of selective overdiagnosis of choriocarcinoma following hydatidiform mole has been discussed, and the suggestion is made that choriocarcinoma is not curable at all.

5. Relationship of hydatidiform mole and choriocarcinoma.

6. The limitations of biologic pregnancy tests.

7. The microscopic appearances. The future of abnormally proliferating trophoblast cannot be predicted by microscopic examination.

8. The relationship between trophoblast and endometrium at the time of implantation. The using of terms such as "invasiveness" and "physiologic malignancy" in descriptions of the changes that occur at implantation is condemned.

9. Nongestational choriocarcinoma.

10. Onset, behavior and spread of choriocarcinoma. The trophoblastic node, extrauterine sites and apparent absence of a primary lesion, the latency of development and of recurrence, the distribution of metastases and spontaneous regressions are described and discussed.

11. Histogenesis, and the evidence for a fetal or a maternal origin of the malignant cells.

12. Analogy with other neoplasms of endocrine origin. It is suggested that chorioma is an "endocrinoma," the neoplasm of the endocrine organ formed in the uterus during pregnancy.

13. The difficulties of diagnosis and treatment.

Notes and News

Deaths.—Samuel Reese Haythorn, of Pittsburgh, director of the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, died Dec. 6, 1949, aged 69. Dr. Haythorn was a member of the American Association of Pathologists and Bacteriologists, of which he was president in 1941; the American Association for the Advancement of Science; the American Society for Experimental Pathology (president in 1932); the American Medical Association; the International Association of Medical Museums (president American and Canadian Section in 1943), and the Society for Experimental Biology and Medicine. He was particularly renowned for his work on silicosis, a field in which he made several excellent contributions to the literature.

Laboratory Training Courses.—Laboratory training courses in the diagnosis of a variety of infectious diseases will be offered by the Communicable Disease Center, Laboratory Division, Public Health Service, Atlanta, Ga. Courses will be offered from January to December 1950 and will be from one to four weeks long. Information and applications may be obtained from the Chief, Laboratory Division, Communicable Disease Center, 291 Peachtree Street, N.E., Atlanta, Ga.

Appointment.—Dr. Jean Paul Wozencraft, New London, Conn., has been appointed associate professor of pathology at the University of Cincinnati College of Medicine. Dr. Wozencraft is a graduate of the university's college of medicine, 1939. He was a fellow at the Mayo Foundation, Rochester, Minn., and held the same rank in the field of tumor pathology at Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York. From 1941 to 1946 Dr. Wozencraft served as pathologist with the Army Air Forces. Since 1948 he has been pathologist at the Lawrence and Memorial Associated Hospitals in New London.

Loeffler Memorial Foundation.—The Hektoen Institute for Medical Research of the Cook County Hospital has allocated $5,000 of its funds for the creation of an Ernst Loeffler Memorial Foundation to support research in honor of the recently deceased research associate of the institute.

Books Received

STREPTOMYCIN AND DIHYDROSTREPTOMYCIN IN TUBERCULOSIS: REPORTS OF RESEARCH INCLUDING STUDIES SPONSORED BY THE AMERICAN TRUDEAU SOCIETY (MEDICAL SECTION, NATIONAL TUBERCULOSIS ASSOCIATION). Edited by H. McLeod Riggins, M.D., and H. Corwin Hinshaw, M.D. Pp. 554. Price, $7.50. National Tuberculosis Association, 1790 Broadway, New York 19, 1949.

This book records one of the finest achievements of American medicine: the appraisal by research procedures of the therapeutic usefulness of streptomycin in clinical tuberculosis.

The subject matter, which was contributed by 58 authors, is divided into thirty-three chapters. Six chapters are devoted to dihydrostreptomycin and its value as an antituberculosis agent, and three chapters are concerned with combined chemotherapy in tuberculosis. Of the thirty-three chapters, fifteen represent reprints of papers published previously. However, for historical reasons and because of the importance of the subject matter, the previously published papers which are included are germane and contribute much to the value of the book.

This book records the story of the development of streptomycin from the time of the announcement of the discovery of the antibiotic, by Schatz, Bugie and Waksman in 1944, to its final recognition as a useful—many believe, an indispensable—adjuvant in the modern treatment of clinical tuberculosis. The book is concerned largely with the results obtained from studies planned for the express purpose of establishing definitive data regarding the usefulness and limitations of streptomycin in clinical tuberculosis. The extensive, cooperative investigations were national in scope, and they were conducted by laboratorians of established reputation and by many of the nation's distinguished phthisiologists.

The comprehensive extent of the investigations, which were inaugurated in November 1946, was made possible by the voluntary efforts of (1) the American Trudeau Society (Medical Section of the National Tuberculosis Association), (2) six of the foremost manufacturers of streptomycin, (3) the United States Public Health Service, (4) university medical schools and affiliated hospitals and (5) many tuberculosis hospitals and sanatoriums.

This is a book written by experts for all concerned with the therapeutic use, in clinical tuberculosis, of streptomycin and dihydrostreptomycin, either or both. Its importance to all who administer these drugs to the tuberculous patient cannot be overemphasized. One of the most important chapters of the book is concerned with the integration of streptomycin therapy and other procedures. Those who may assume that the newer chemotherapy makes older and conventional methods of treatment obsolete will find this chapter most rewarding.

As is true of many multiauthored medical books on a single subject, this book suffers from minor duplications and from a diversity of literary style. The excellent reproductions of the many roentgenograms is a technical triumph not always achieved when this type of illustrative material is reproduced on the printed page. Unfortunately, descriptive legends were omitted from a considerable number of the roentgenographic illustrations, thus reducing their effectiveness.

In spite of the comprehensive nature of most of the clinical investigations recounted, the respective contributors have maintained a commendable circumspection in assessing the significance of their observations. All seem to agree that

the accession of knowledge is never final and that additional information regarding streptomycin as an antituberculosis agent will continue to contribute to the usefulness of this drug.

The editors and compilers, Drs. Riggins and Hinshaw, should be congratulated for having accomplished in a commendable way a most difficult task in the coordination of the respective investigations and in making available so promptly the results of the study.

The volume is well indexed, well printed and substantially bound.

DIE DOPPELSEITIGEN NIERENKRANKHEITEN MORBUS BRIGHTII: EINE NEURALPATHOLOGISCHE BETRACHTUNG (BILATERAL RENAL DISEASE: A NEUROPATHOLOGIC CONSIDERATION). By Prof. Dr. Wilhelm Nonnenbruch. Pp. 210, with 15 illustrations. Price, bound, 21.20 marks; unbound, 18 marks. Ferdinand Enke Verlag, Hasenbergsteige 3, Stuttgart, W. Germany, 1949.

This book concerns extrarenal symptoms of bilateral diseases of the kidneys. After an introductory review of the anatomy and the physiology of the kidney, the author, often using case histories, discusses nephrosis, nephritis, hypertensive cardiovascular disease ("essential benign and malignant hypertension") and the kidney in pregnancy. The hepatorenal syndrome is also mentioned.

The book is written principally from the point of view of a clinician who firmly believes in the neurogenic origin of hypertension. He regards hypertensive cardiovascular disease ("essential hypertension") as the result either of a primary disturbance or disease of the midbrain or of a "reactive irritation of the midbrain," the result of an abnormal reflex mechanism. There may be either no anatomic lesions within the central nervous system or blood vessels or several changes in these structures.

Because of the assumption that the cause of hypertensive cardiovascular disease lies within the central nervous system, the author does not believe in sympathectomy as a cure. He quotes statistics of German investigators which indicate that in about half of the patients the blood pressure did not drop after Peet's operation. The book is written on the basis of a large clinical experience, but there is practically no discussion of the morphologic changes occurring in the kidney or the blood vessels. The possible relation of chronic pyelonephritis to hypertension is not mentioned, probably because, the primary subject under discussion being bilateral diseases of the kidney, no consideration is given to the fact that chronic pyelonephritis is just as often bilateral as unilateral. The reviewer was often impressed by the author's rather philosophic attitude which culminates in the statement that "the gifted layman, as the result of intuitive appreciation of the whole, often is more successful than the faithful disciple who does not grasp the 'spirit' of medicine."

PHOTOELECTRIC METHODS IN CLINICAL BIOCHEMISTRY. By G. E. Delory, M.Sc., Ph.D., assistant professor of biochemistry, University of Manitoba, Winnipeg, Canada. With a foreword by Prof. E. J. King, M.A., Ph.D., D. Sc. Pp. 90, illustrated. Price, 15 shillings. Hilger and Watts, Ltd., 98 St. Pancras Way, Camden Road, London, N.W.1, England, 1949.

The simplicity and sensitivity of colorimetric methods, along with a precision satisfactory for most purposes, have led to their being extensively developed for chemical analysis of body fluids. Over the past few years photoelectric colorimeters of many types have steadily replaced the direct vision Duboscq type of instruments for this purpose. Although the advantages of these newer instru-

ments are many, they are a bit more complex and, therefore, must be used with understanding and care to avoid disturbing errors.

The objectives of this book, according to the authors, are threefold: "(1) to give in as simple fashion as possible the theoretical background needed for intelligent use of photoelectric colorimeters, (2) to give descriptions and instructions for the use of the two instruments manufactured by Messrs. Hilger and Watts Ltd. . . ., and (3) to give practical details for the colorimetric procedures most commonly undertaken in clinical biochemical laboratories." The authors have fulfilled the demands of these objectives in a creditable manner. The theoretic treatment is brief but adequate for the purpose. The section describing in detail 22 well selected methods is well organized. The principle of each method is briefly stated, followed by a list of reagent requirements and a "blow by blow" description of the procedure. Calculations, notes and a typical calibration curve are included for each method. In many cases the quantity of blood required is so small that it can be collected readily by finger puncture.

Although the two sections just described will be of general usefulness, the section (10 pages) describing Hilger instruments will be of interest only to those who have this type of instrument or who are thinking of purchasing such an instrument.

The book will be of interest to the laboratory supervisor as a clear and concise treatment of the subject, and to the laboratory technician as a source of explicit and detailed directions of 22 colorimetric methods adapted for the photoelectric colorimeter.

CONTRIBUCIÓN DEL COEFICIENTE CITOLÓGICO A LA FISIOLOGIA Y PATOLOGIÁ DE LA CORRELACIÓN HIPOFISOTIROIDEA (THE CYTOLOGIC COEFFICIENT AS A MEANS OF DETERMINING THE NORMAL AND ABNORMAL RELATIONSHIP OF THE HYPOPHYSIS TO THE THYROID). By Estanislao Del Conte, head of the division of histophysiology in the Institute of General Anatomy and Embryology of the Faculty of Medical Sciences, Buenos Aires (Argentina). Monograph. Pp. 87, with 16 plates and 34 illustrations. Librería y Editorial "El Ateneo," Florida 340—Córdoba 2099, Bueños Aires, 1949.

Sections of thyroid gland for histologic study were prepared from frozen and dried material by the Altmann-Gersh method. Vacuoles in the colloid and cells of the thyroid gland are regarded as artefacts produced by the ordinary methods of fixation. The colloid is absorbed from the follicles transcellularly and not intercellularly. On the basis of experimental evidence that during activation of the thyroid gland droplets of colloid rapidly accumulate within the lining cells of the follicles, the author gaged the thyrotropic activity of serums by means of the cytologic coefficient. The latter represents the ratio of the total number of intracellular colloid droplets of a given number of follicles to the sum of the mean diameters of the same follicles. In standardizing this procedure on rats and guinea pigs with the use of a pituitary extract containing the thyrotropic factor, the author found guinea pigs to be far more sensitive than rats and the coefficient a more delicate index of thyrotropic activity than any procedure devised to date. Employing human serum freed of thyroxin by Fellinger's procedure on young fasting guinea pigs as test objects, he was able to demonstrate a reduction in the amount of thyrotropic hormone in classic exophthalmic goiter, with values returning to normal after thiouracil therapy or thyroidectomy. In cases of hyperthyroidism with exophthalmos, local edema or post-thyroidectomy recurrence the thyrotropic values were elevated, returning to normal after treatment with thyroxin.

GYNAECOLOGICAL HISTOLOGY. By Josephine Barnes, M.A., D.M. (Oxford), M.R.C.P. (London), F.R.C.S. (England), M.R.C.O.G.; assistant, Obstetric Unit, University College Hospital, London; assistant obstetrician and gynecologist, Elizabeth Garrett Anderson Hospital; surgeon, Marie Curie Hospital. Membre correspondent, Société Française de Gynécologie. Pp. 242, with 162 illustrations. Price, $6.50. Grune & Stratton, Inc., 381 Fourth Avenue, New York 16, 1949.

This slender volume is essentially an atlas of gynecologic and obstetric histology, normal and pathologic. The author first planned the book while preparing a catalog of gynecologic slides for students. All the 162 illustrations, with one exception, are photomicrographs of actual slides at varying magnifications. Apart from the legends for the illustrations there is a brief relatively schematic text, which makes no attempt to deal with controversial or recondite material. The topics covered are the corpus and cervix uteri, the fallopian tube, the ovary, the vagina, the vulva and pregnancy. The photographs show variation in clarity and excellence but on the whole are representative, adequate and sometimes superlatively good. The text is clear but possibly oversimplified.

The book is of considerable value for a gynecologist, an obstetrician or a surgeon, whether resident or in practice, who wishes to have readily available a collection of relevant material in photographic form.

PHYSIOLOGY IN DISEASES OF THE HEART AND LUNGS. By M. D. Altschule, assistant professor of medicine, Harvard Medical School; visiting physician and research associate, Beth Israel Hospital; director of internal medicine and of research in clinical physiology, McLean Hospitals. Pp. 368. Price, $5. Harvard University Press, Cambridge 38, 1949.

This book was written, as the author stated, to satisfy requirements of third and fourth year medical students who felt the importance of refurbishing on the details of the fundamental sciences of the preclinical years—to appreciate and understand clinical phenomena. Thus, the various diseases of the heart and the lungs are presented from the point of view of abnormal function. A particularly interesting and timely chapter deals with congenital defects of the heart. It is brought out that catheterization of the right side of the heart and application of surgery have made available data on the physiology of congenital heart disease formerly not obtainable. What has been learned in this respect during the last few years is given in a concise fashion. The bibliography which follows each chapter is excellent. This is a valuable handbook not only for third and fourth year medical students but for all who wish to obtain a clearer concept of the underlying abnormal function of diseased structures.

CLINICAL BIOCHEMISTRY. By Abraham Cantarow, M.D., professor of biochemistry, Jefferson Medical College; and Max Trumper, Ph.D., Commander, H(S), U.S.N.R., and lecturer in clinical biochemistry, and basic science coordinator, Naval Medical School National Naval Medical Center, Bethesda, Md. Fourth edition. Pp. 642, with 38 illustrations. Price, $8. W. B. Saunders Company, 210 W. Washington Sq., Philadelphia 5, 1949.

CANCER. By Beka Doherty. Pp. 327. Price, $3. Random House, Inc., 457 Madison Ave., New York 22, 1949.

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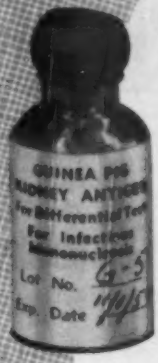

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1. I. DAVIDSOHN, K. STERN and CHIYO KASHIWAGI: The Differential Test for Infectious Mononucleosis; Abraham Levinsohn Anniversary Volume Studies in Pediatrics and Medical History, Froben Press, Inc., New York 1949.

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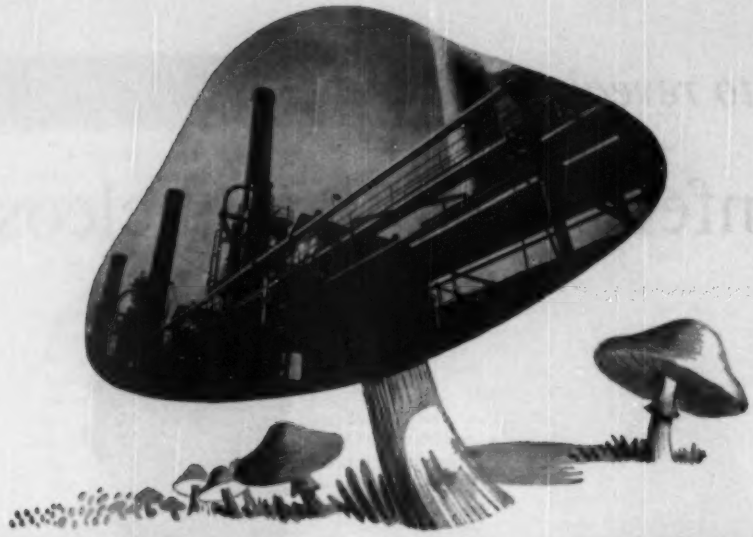

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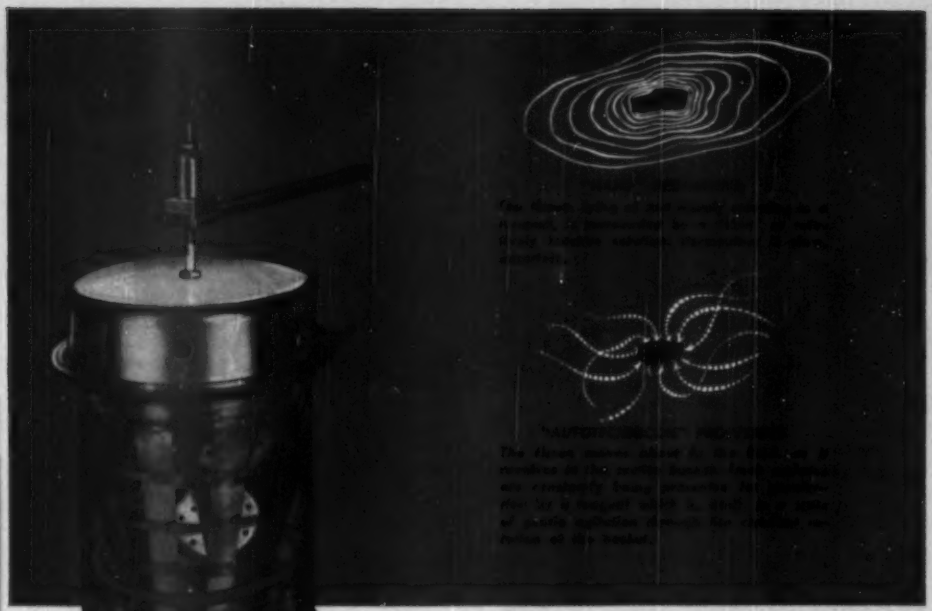

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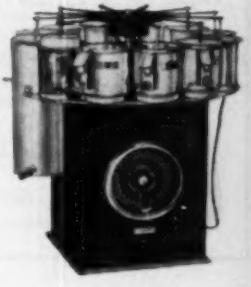

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